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A Photometric Method of Studying the Control of Handedness by the Cerebral Cortex of the Rat

Donald K. Gucker

A PHOTOMETRIC METHOD - GUCKER

A PHOTOMETRIC METHOD OF STUDYING THE CONTROL OF HANDEDNESS BY THE CEREBRAL CORTEX OF THE RAT

By

Donald K. Gucker

A Thesis

Submitted in Partial Fulfillment of the

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This thesis, directed and approved by the candidate's committee, has been accepted by the Graduate Committee of the University of New Mexico in partial fulfillment of the requirements for the degree of

MASTER OF ARTS

E. L. Castetter
DEAN

DATE May 25, 1959

Thesis committee

Thorne Peterson
CHAIRMAN

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"A science of the brain must point out the functions of its elements. A science of the relations of mind and brain must show how the elementary ingredients of the former correspond to the elementary functions of the latter."

William James, 1890

TABLE OF CONTENTS

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

INTRODUCTION

In 1948 Hilgard wrote, "It is a blot upon our scientific ingenuity that after so many years of search we know as little as we do about the physiological accompaniment of learning." (Hilgard, 1948, p. 481) Today the situation remains unchanged; no one has been able to identify the change or modification occuring in neural tissue that permits learning and retention. The statement that structural modification occurs with learning is an assumption, but one that enjoys almost universal acceptance. Hilgard goes on to say that, "one of the most crying needs is for a crucial experiment identifying specifically a change occuring in neural tissues. . . as learning takes place." (Hilgard, 1948, p. 481) This thesis describes the first in a series of proposed experiments designed for identification of such a change.

Among the problems involved in such a program is the approach to be taken. The concept of structural change is many-sided and may involve change at a chemical level, a visible cellular level, or perhaps at some other unknown level. In addition to the nature of the change the problem of degrees of localization of learning has yielded various viewpoints that must be reckoned with.

Concerning the chemical approach, Rosenzweig, Krech,

and Bennett (1958) ask the question, "What changes in the nervous system accompany learning? Though learning is generally considered to involve structural changes, and though the search for such changes has been the preoccupation of many experimenters and theorists, the complexities of the anatomy of the nervous system seem so far to have prevented the detection of such changes. A biochemical analysis which could integrate changes over thousands of neural units might provide an entering wedge to the solution of this problem. Further analysis might then focus more narrowly on the exact sites of change." One difficulty with this statement is the assumption that thousands of neural units are involved in a learning situation. At its present state of development the chemical approach is a gross one, and admittedly faces the problem of specialization as seen in localization of function.

Seeking a visible cellular change also presents formidable problems. Wyckoff and Young (1956) state, "The development of adequate methods for cutting thin tissue sections and improvements in techniques of fixation now make it possible to study the relations between the cells within the nervous system at the high resolutions of the electron microscope; the detail thus seen is bewildering in its complexity." In this dense forest of structure one wonders what type of change to seek. It is not im-

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nervous system accompany learning? Though learning is generally considered to involve structural changes, and though the search for such changes has been the preoccupation of many experimenters at all levels, little is known of the anatomy of the nervous system which would have prevented the detection of such changes. A histochemical analysis which could demonstrate changes in the sands of neural units might provide an alternative route to the solution of this problem. Further analysis might then focus more narrowly on the exact sites of change.

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Seeking a viable solution to this presents formidable problems. Wyckoff and Young (1956) state, "The development of adequate methods for cutting thin tissue sections and improvements in the fixation of tissue make it possible to study the relations between the units within the nervous system at the ultrastructural level by electron microscopy for tissue thus seen is beautiful in its complexity." In this dense thicket of entities one wonders what type of clarity is...

possible for almost any of the numerous neural structures to participate in the modification, but theory and a scrap or so of evidence has narrowed "the field" down a bit. The structures that are encompassed in the concept of the synapse seem to appear in modification theory more often than other neural structures, and this seems to be due to the fact that it is easier to envisage change at multiple neural junctions than at other places in the system. Hebb (1949) postulates a thickening of fibers and/or enlargement of synaptic knobs as two possibilities. Eccles (1953) has illustrated at the level of the cord that the physiological properties of the synapse are modifiable depending on use and disuse. Specifically he modified the transmissive abilities of the synapses by controlling the amount of use they were subjected to and thus lent support to the theory of change at the synaptic junction. Eccles explains his evidence in terms of postulates concerning change in size of boutons with use and disuse (1953). Learning theory has utilized synaptic structures with little regard for the fact that our information concerning these structures came from investigations of spinal neurons. Only recently has evidence been presented concerning the presence of similar structures in the cerebral cortex.

Smythies, Gibson, and Purkis published a paper on "The Distribut ion and Morphology of Boutons Termineaux

in the Human Cerebrum." (1957) Their study seems to establish the presence of boutons in the cortex, but their conclusions concerning their distribution seem questionable, considering that a silver stain was used. The important point here is that the boutons do seem to exist in the cortex; this at least lends the air of feasibility to theories which utilize boutons as a mechanism involved in learning.

Recently Palay (1956a) demonstrated electron micrographs of boutons present in the medulla. Under the tremendous magnification of the electron microscope a complex typical internal structure is revealed. This structure is characterized by the presence of mitochondria and small vessicles. In a publication concerning synapses in the central nervous system Palay states: "In other synaptic fields, such as the molecular layer of the cerebellar cortex and in the cerebral cortex, formations with this essential structure also appear. In these regions, the axon terminals are small and contain only a few mitochondria and a cluster of vessicles located near a thickened limiting membrane in apposition to a typical dendritic process." (Palay, 1956b, p. 198) His study utilized the brain of the albino rat.

The staining of boutons is difficult, patchy, and unreliable; consequently any attempt to use their number or their size as an index of change would face grave

difficulties. Eccles, in his discussion of plasticity, perhaps points to a method of avoiding the "bouton problem." He states,

"The most probable postulate for post-tetanic potentiation is that the presynaptic impulse becomes a more effective synaptic excitor (or inhibitor), because repetitive stimulation temporarily alters the spatial relationship of the synaptic knobs to the post-synaptic membrane; for example, the knobs may become larger and/or in closer apposition thereto. The volume changes observed in repetitively stimulated giant axons (Hill, 1950) may be expected to occur also in the non-medullated presynaptic fibres during and after repetitive stimulation, for the uptake of water in considerable part at least is determined by the flux of ions that is associated with the nerve impulse. The relatively large surface to volume ratio associated with the very small diameter of these fibres (probably no more than 0.2 to 0.3 μ) would lead one to expect a relatively greater swelling and possibly a more rapid time-course of the swelling and recovery therefrom. Furthermore, given uniform elastic properties of the membrane, the synaptic knob, having a larger radius of curvature than its attached fibre, would be expected to swell more readily than this fibre in response to an increase in turgor of the system; i.e., there would be a flow of axoplasm from the swollen fibre to increase

further the swelling of the knob.

If it is assumed that the uptake of water per square centimeter of surface is the same as in the gian fibre (where it is rather more than 10^{-5} cc. per sq. cm. of membrane for 10,000 impulses), then there is a surprisingly large swelling of the fine presynaptic fibre and knob. A 0.3 diameter fibre would more than double its volume, swelling to 0.46 μ diameter, and a 1.0 μ diameter (spherical) knob would swell to 1.2 μ." (Eccles, 1953, pp. 198-199)

Consequently we might conclude that if we cannot examine boutons because of difficulties in staining, then perhaps changes in turgor may be reflected in more stainable structures such as fibers and cell bodies. If internal pressure is increased to the point of swelling the fiber, then it is not unlikely that small concomitant changes in the volume of the cell body may occur. Even if these changes are only in the order of a tenth of a micron, but occur in a number of cells, they might be detected by a sensitive instrument such as Hill (1950) used in measuring small volume changes in squid axons. It is this general line of reasoning that this experiment follows. Before entering the particular nature of this investigation, however, one other point must be mentioned. The physiological psychologist must decide before attempting to investigate learning histologically, where

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in the nervous system this learning is located. Sperry (1958, p. 401) summed up one point of view when he wrote, "Cortical lesions produced initially in the expectation that whole blocks of memory and categories of experience and behavior would be wiped out proved in many cases to have so slight an effect on functional organization and memory as to tax the ingenuity of the investigator to detect any behavioral defects. The question of the locus and nature of the memory trace or engram became recognized, through the work of Lashley, in particular, as one of the most baffling of all neurological problems. Almost any engram scheme that might be expressed within the framework of the traditional approach to cerebral integration, i.e., in terms of specific fiber connection, seemed to be ruled out."

Lashley (1950, p. 477) treated the subject of localization most directly when he wrote, "all the cells of the brain are constantly active and are participating, by a sort of algebraic summation, in every activity. There are no special cells reserved for special memories." The alternative school of thought, that is there are specific loci of learning, has recently been defended by Hebb in his book, The Organization of Behavior (1949). Hebb states, "The assumption we must accept is that the memory trace, the basis of learning, is in some way structural and static." (p. 12) There are two primary obstacles to

the acceptance of this assumption; the first being the work of Lashley and the second is the work of the Gestalt psychologists. Kohler has developed a theory of electrical fields in the brain to account for perception and learning. Gestalt investigators emphasize pattern rather than locus, and it is only fair to say that they have considerable experimental data for support. Hebb spends the major portion of his book trying to show that their conclusions are not justified by their evidence. Our primary concern here is with the work of Lashley, because it is much closer to the subject at hand than the work of the Gestaltists. Hebb (1949, p. 13), when speaking of Lashley's work, points out, "that the removal of blocks of the rat's cerebral cortex does not affect habits selectively. If one habit is affected, others are also. From this Lashley has concluded that memory traces are not localized in the cerebral cortex, but himself has pointed out another possible interpretation. His evidence is consistent with the idea that the trace is structural, but diffuse, involving, that is, a large number of cells widely spaced in the cortex, physiologically, but not anatomically unified." "This is not, consequently, crucial evidence for or against the notion of structural traces in the cortex." If specific loci are not important, then how can repeated sensations that reinforce one another result in the relatively permanent effect we call learning? If we

accept a hypothesis concerning reverberatory activity among neural chains with no specific loci, it becomes difficult to imagine how these activities would not interfere with each other and how they could survive violent cortical storms such as grand mal epilepsy or cerebral concussion. As Hebb states, "a pattern of activity not dependent on structural changes for its permanence--this seems in the highest degree unlikely." (Hebb, 1949, p. 13)

We cannot strictly prove Lashley's statement concerning "no special cells" to be incorrect. However, I have cited some evidence and thought that does not support his conclusions.

Practically speaking, it is easier to search in some specific place than any place in general by some specific means. Any general approach must contend with the microscopic size of the cortical elements presumably involved in learning, and also with the concept that different instances of learning may involve different types of change. Analytical devices available for such general approaches do not take these questions into account, and consequently on a practical level one finds advantages in searching for a structural change in a specific locus.

In the gross pursuit of the structural trace, one encounters the problem of cortex versus sub-cortical structures, and in this delineation we find some of Lashley's work useful. It has been held that memory traces

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are first formed in cortex and then transferred to sub-cortex. This notion results from the greater instability of recently-formed habits than of old habits, and from the assumption that consciousness is a cortical function. Old habits become automatic and are performed without conscious control; consequently they are not located in the cortex. This reasoning has been discredited by Lashley's work on the discrimination of brightness in the rat. Removal of the striate cortex abolishes the brightness habit completely, but with new practice the animals are able to relearn the habit. Tremendous overlearning in a group of animals produced no change in the results. "The long, overtraining did not eliminate the participation of the cortex. . . . After animals, lacking the visual cortex, have learned a brightness habit, any other part of the cortex may be destroyed without disturbance of the habit. Apparently no other part of the cortex takes over the learning function." (Lashley, 1950, p. 466) Lashley showed that the particular sub-cortical structure involved does not participate in the learning as long as the necessary cortex is intact. He sums up his experimental work in this area, "if the cerebral cortex is intact, the associative connexions of simple conditioned reflexes are not formed in the sub-cortical structures of the brain." (Lashley, 1950, p. 467) There have been numerous studies concerning the possibility of condi-

tioning occuring at lower levels in the central nervous system (Morgan & Stellar, 1950, pp. 439-451), but they are unimportant here because intact cortex is prepotent; consequently, it is here that our interest lies.

Specifically, our question boils down to this: where are the cortical loci of learning and what specific modifications are concomitant with this learning?

Handedness in the rat has been selected as a behavioral function subject to modification by practice (Peterson, 1934). In searching for a structural correlate handedness yields several advantages. First, it is easily determined by a food reaching situation (Peterson, 1934). Second, it is modifiable by practice and therefore subject to a form of learning. Third, its cortical representation has been highly localized in the rat (Peterson & Gucker); and lastly, it has a unique double cortical representation (when the non-preferred hand is included) with an area in each hemisphere. One of these areas controls the preferred limb and the other controls the non-preferred limb. The simultaneous existence of these two areas, one subject to practice and the other not, offers a basis of comparison unique in the search for a structural correlate. The objective of this investigation is to compare histologically, the preferred cortical area with its homologue and seek a structural difference in animals with known hand preference.

Considering our lack of knowledge concerning the functional significance of neuro-anatomical elements, a rather unique method has been devised for histological observation and deserves brief mention at this point. As stated previously, one feasible change is of size due to increased turgor pressure in a closed system.

Hill (1950) detected and measured small changes in the size of the giant axon of the squid by optical means. He measured the quantity of light entering a photocell after passing through an optical arrangement in which the axon was inserted. This investigation uses the same principal but apparatus that is quite different from Hill's. This method does away with the need for subjective evaluation of structural elements and thereby meets the objections voiced by Lashley and Clark (1946) concerning such methods of observation. Using this apparatus in conjunction with stains selective for a particular cortical element allows inquiry into the parts played by these elements in the role of learning.

Consequently, this study is designed to compare the amount of light impedence occuring in the cortical areas controlling the preferred and the non-preferred limb in the rat, in an attempt to relate optical density of structure to preferential handedness.

METHOD

Subjects

Ten male hooded rats were selected after testing in the Peterson food-reaching situation. Any rat that did not meet the requirements of a single hander was removed at this point. Single-handed rats are defined for purposes of this study as those taking not more than two percent of total reaches with the non-preferred hand. A total of thirty-two rats were tested in order to find ten such single-handed animals. Table I presents the reaching records of these animals. It will be seen that there were five right-handed and five left-handed rats.

Table I -- Reaching records

Rat No.	Left reaches	Right reaches	Handedness
1	0	450	R
2	0	450	R
3	0	450	R
4	2	448	R
5	4	446	R
6	450	0	L
7	450	0	L
8	450	0	L
9	447	3	L
10	442	8	L

Apparatus

The primary piece of apparatus is an ultra-sensitive and stable densitometer with its associated equipment. The densitometer utilized a vacuum phototube as a light receptor. This phototube was placed in a 35 mm. camera body mounted on the third ocular of an A/O trinocular microscope. A slide placed on the mechanical stage of the scope was subjected to a light beam of variable diameter coming up from the illuminator and passing through the sub-stage condenser. The Sleige and Khune microscope illuminator was voltage-regulated by a Sola #30886 regulator operated at full load. Instrument stability seemed adequate as evidenced by no observable fluctuations in the indicator circuit during operation. After passing through the tissue the light beam enters the 10 x objective of the scope and passes through its usual optical system. The photocell receives the beam after it enters the camera objective. The cathode is placed slightly above the focal plane of the camera so that the image will be slightly out of focus and thus negate any local variation in the image or cathode surface. The phototube is mounted in a metal box that is attached to the camera, and it is grounded to the shielded cable that connects the phototube to the amplifier.

A densitometer is an instrument which measures the amount of light transmitted by a substance, and the photo-

Apparatus

cell with amplifier stages described here constitute such an instrument. The d.c. amplifier circuit is one published by RCA (Mark, 1956) and designed specifically for use as an ultra-sensitive wide-range unit. A schematic is presented in Figure 1. The photocell constitutes one arm of a bridge circuit that uses R_1 as a balance. The voltage reference and regulator circuit has been modified to include a 6627 tube and thus provide a closer degree of reference than the OC3/VR105 originally specified by RCA. The B+ voltages are supplied by a Tektronix 160A power supply, thus providing a B+ of 225 volts when line voltage varies between 105-125 volts. The filament 6.3 VAC also came from the power supply but was not regulated. Since a regulated B+ was used in conjunction with the reference circuit in the amplifier, excellent drift-free stability was obtained. Several prototype amplifiers were constructed before a suitable instrument was produced. It proved necessary to use high quality precision 1 percent resistors throughout in order to reduce all possible sources of noise. It was found necessary to shield the entire unit in a metal case; otherwise a person near the instrument caused deflection of the galvanometer.

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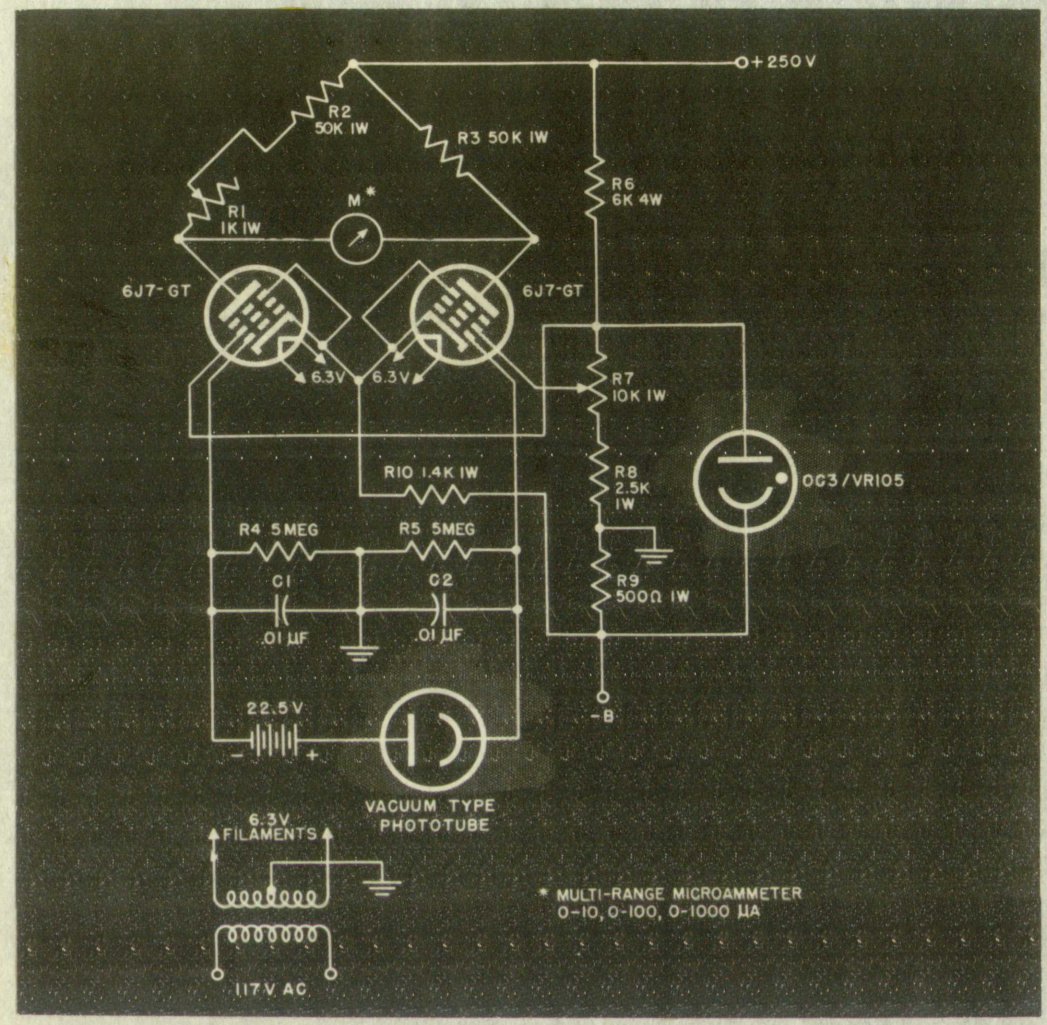


Figure 1 -- Schematic of Densitometer

The difference in d.c. potential on the plates of the 6J7's is applied to the floating inputs of a Kintel model 204 Electronic Galvanometer. This instrument provides a d.c. chopper amplifier stage with an Aryington shunt at the input. This is a completely transistorized unit that exhibits high stability and sensitivity. The shunt was set so that a difference of 100μ a applied to the input

yielded a full scale deflection. Balance was obtained by using R_1 as a coarse balance and adding a fine control in series. A rough idea of the extreme sensitivity provided by the amplifier is found in the statement, "The sensitivity of this circuit will provide a usable meter deflection for phototube currents as low as .0001 μ a." (Mark, 1956, p. 78) The circuit provides an essentially linear relation between meter current and illumination.

The instrument reflects the amount of light transmitted, primarily by the stained structures in the tissue, as a quantitative reading on the galvanometer, thus providing a measure of the density of stained elements.

PROCEDURE

Tissue Preparation

After taking the last 50 reaches out of a total of 450 in the handedness test situation, the animal was sacrificed under ether, the brain removed and placed into 10% formol. After at least 24 hours in the fixative, serial 40 sections were cut by the freezing technique and mounted with Mayers Albumen, four to a slide. The cortical area sectioned was determined by previous localization studies (Rivoire, 1957; Peterson & Gucker, in press) and included that between the anterior pole of white matter and the dorsal convexity of the caudate nucleus. Usually 16 to 24 serial sections 40 μ thick encompassed this area. The hemispheres of a single brain were sectioned separately, due to a lack of bilateral symmetry in the location of the white matter and caudate nucleus, with the left always being sectioned first. Small variations in section thickness are allowed to vary at random due to the difficulty in control in the freezing technique. Since five left-handers and five right-handers are used, any influence due to sectioning of the left hemisphere first is controlled. The freezing technique is preferred in this investigation because it does not involve the shrinkage and difficulties involved in the celloidin and paraffin methods. After mounting on slides

both hemispheres are placed in the oven at 35-40°C. for at least 24 hours. The two hemispheres of each brain are treated as much alike as possible. Different brains may be treated differently, but this produces no loss of control since the comparisons are to be made between the two hemispheres of a single brain and not between brains.

After drying in the oven, both hemispheres are placed back to back in an alternate fashion in a ten-slot staining dish. The purpose of this arrangement is to subject both hemispheres to the same staining conditions. The sections are then stained by the Kluver Barraer technique for fibers and cell bodies. A detailed description of this method is included in the appendix. After staining, the slides are mounted with balsam and number two cover glasses.

Densitometer Observations

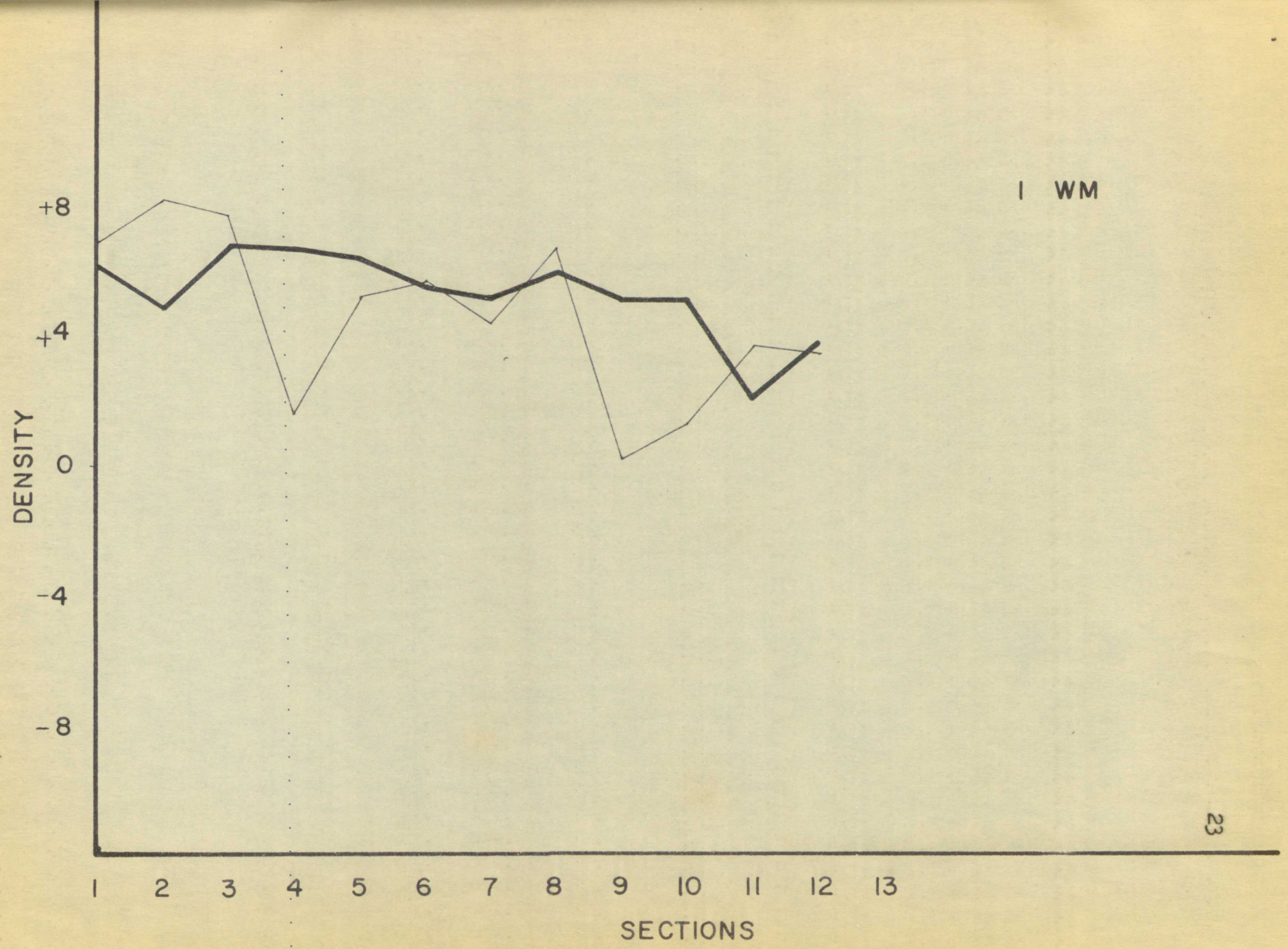
The observers, GP and KE, were unaware of the handedness of the rats involved in the study except for knowing that five were left-handers and five right-handers. The identification marks on the slides gave no indication of handedness. Each observer made two observations on each slide, one in the white matter and the other in layer VI of the cortex. The densest spot in the white matter was searched for and recorded because several preliminary cases suggested a relation between density of fibers and

handedness. With the Kluver stain a band of lightly stained fibers occurs just above the white matter in layer VI. One observer, KE, searched for the lightest area in this band and recorded that density; and GP selected a random sample from this area, thus giving two types of readings in layer VI. Since fibers stain lightly in this band, the comparative influence of cell bodies is probably rather strong. An arbitrary slide was selected, usually in the left hemisphere, and the bridge balanced to a zero setting on the galvanometer. The slides in that hemisphere and the other derive their absolute readings from this relative zero. The zero point is different for not only each rat, but also between white matter and layer VI readings in the same animal. The extreme sensitivity of the instrumentation required this reset procedure. The diameter of the light beam used for readings in the white matter was approximately 240 μ, and that used for layer VI was approximately 560 μ. The diameter of the beam was set before each set of readings and not changed until each particular set was completed.

RESULTS

Graphs are presented, ten for the white matter and ten for layer VI, that compare the density of the preferred hemisphere with that of the non-preferred hemisphere. The heavy line represents the density of the various sections of tissue, starting at the left of the baseline with the beginning of the white matter and moving to the head of the caudate nucleus at the right, of the preferred hemisphere the thin line represents the same density relations for the non-preferred hemisphere.

The graphic results of KE were so similar to GP's that computation and graphic material presented here are those of GP. One reason for the selection of his data is that the layer VI material resulted from a more or less random sampling of the cortical area, while KE selected the lightest spot available in the area.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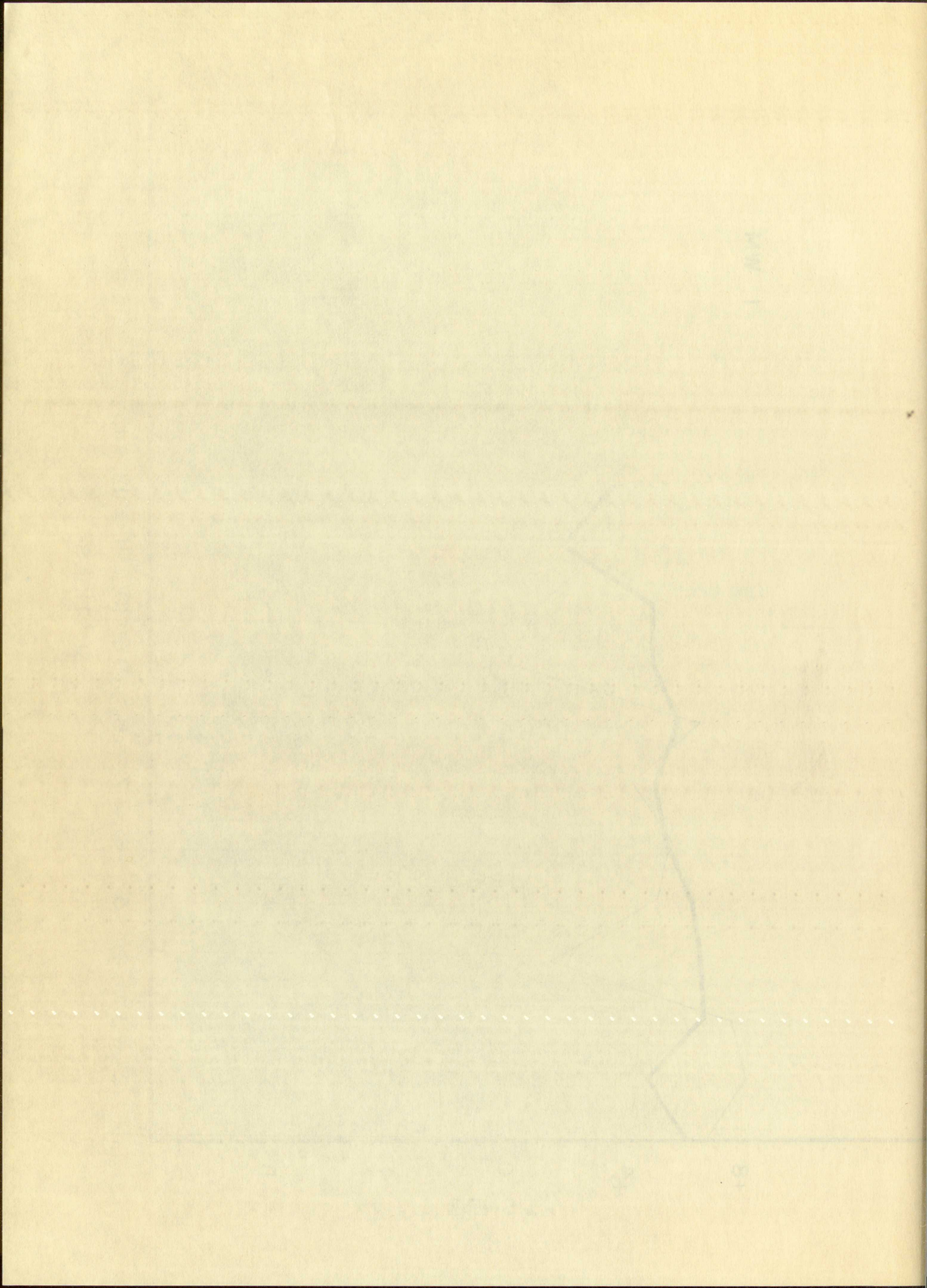

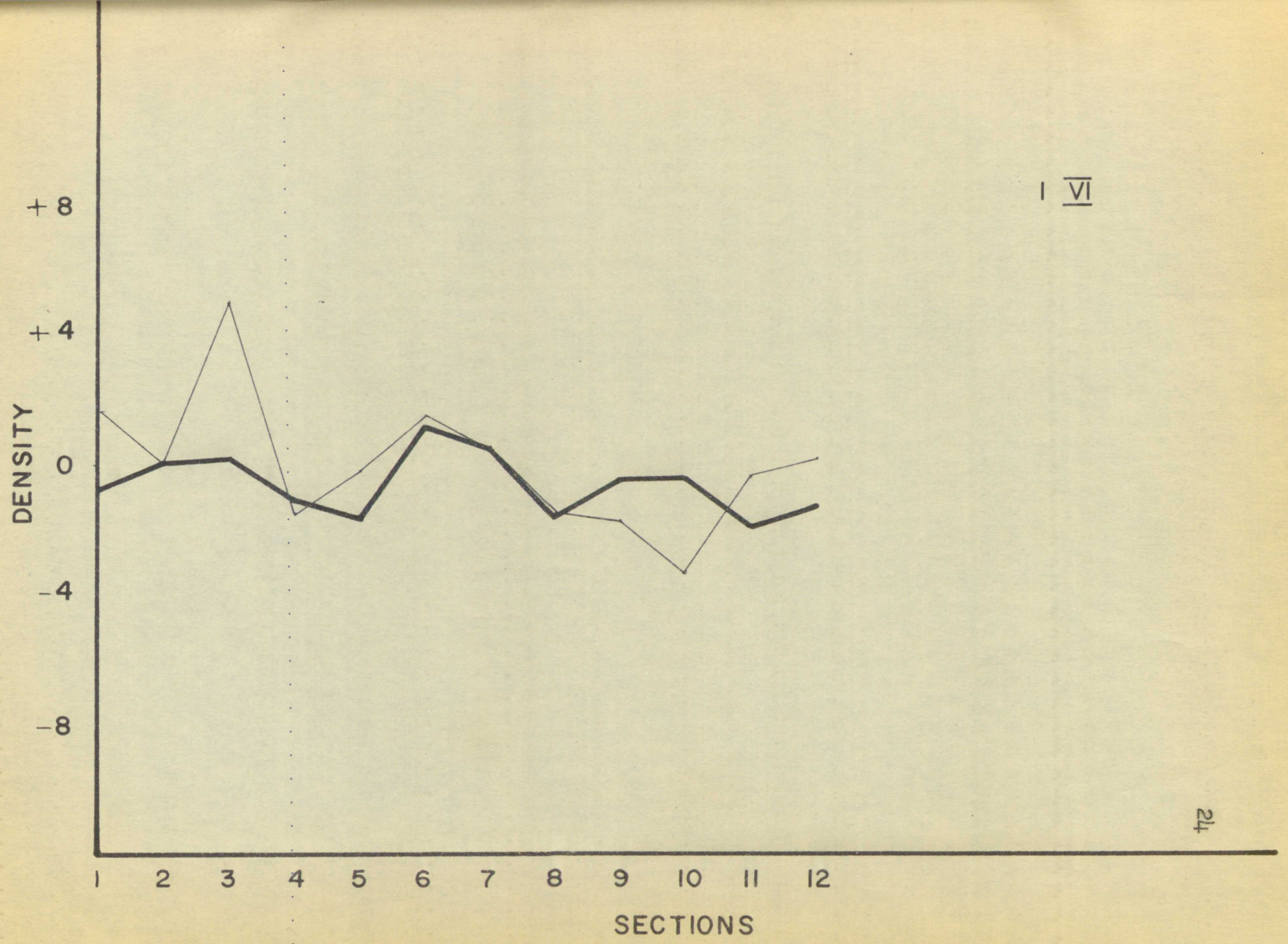
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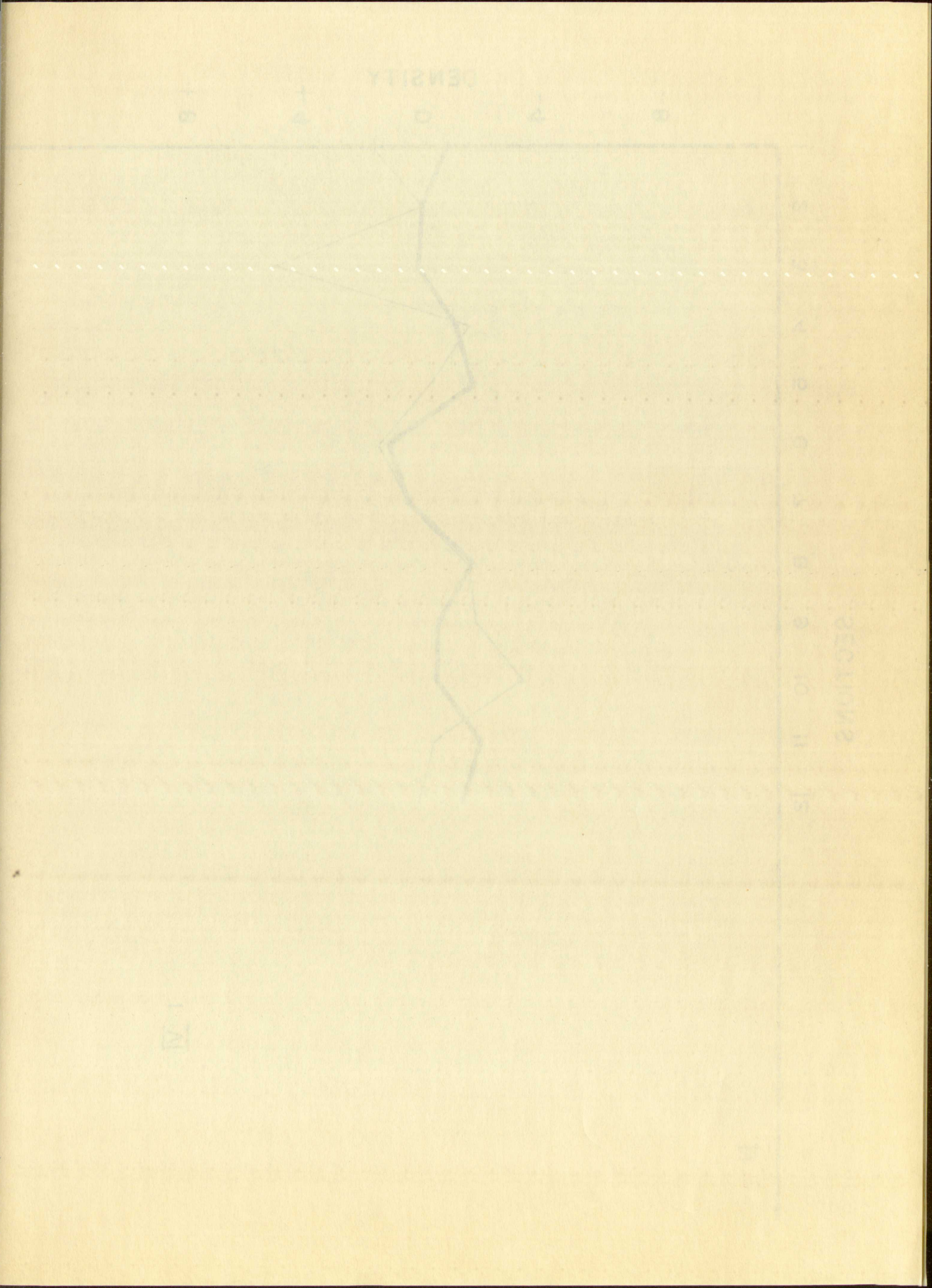

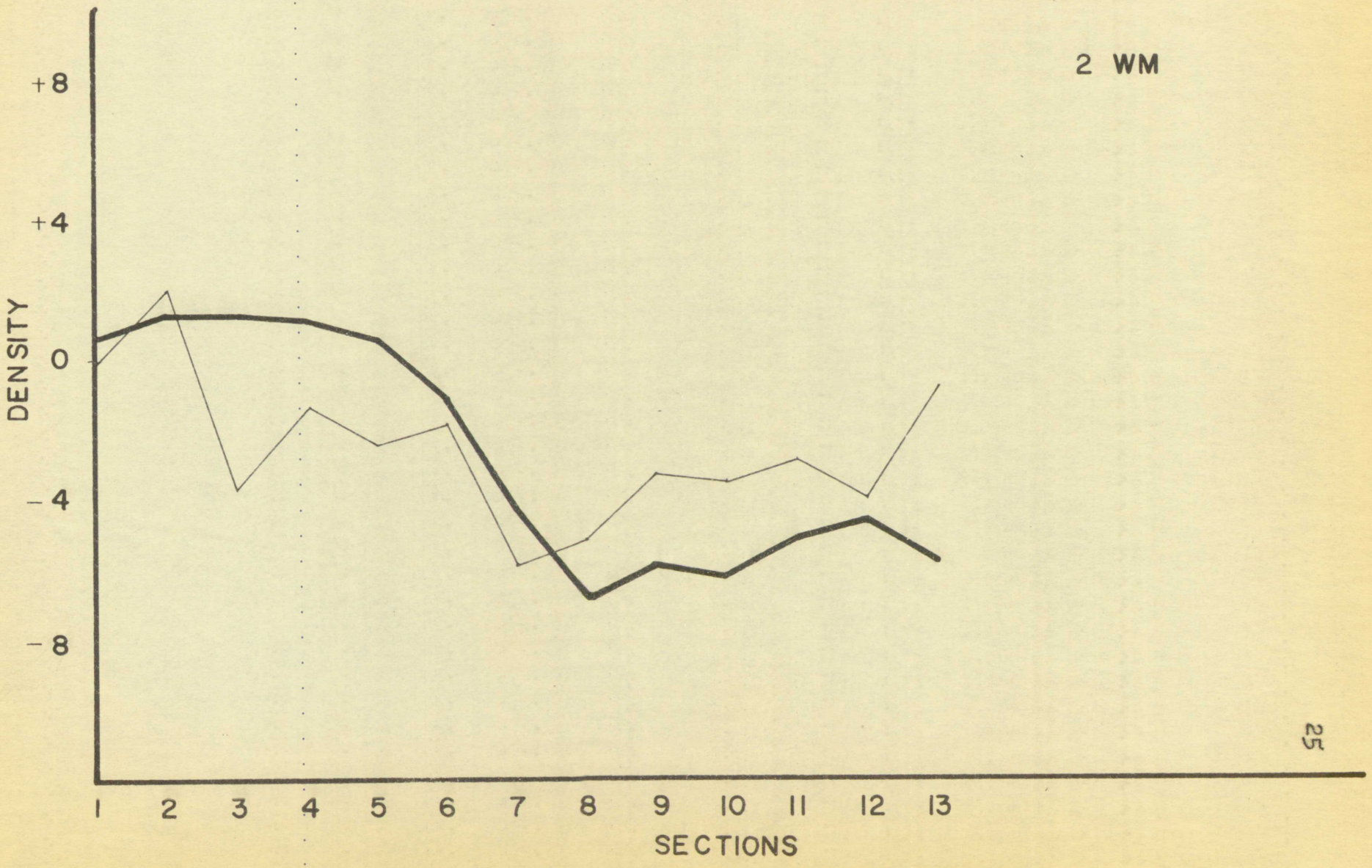
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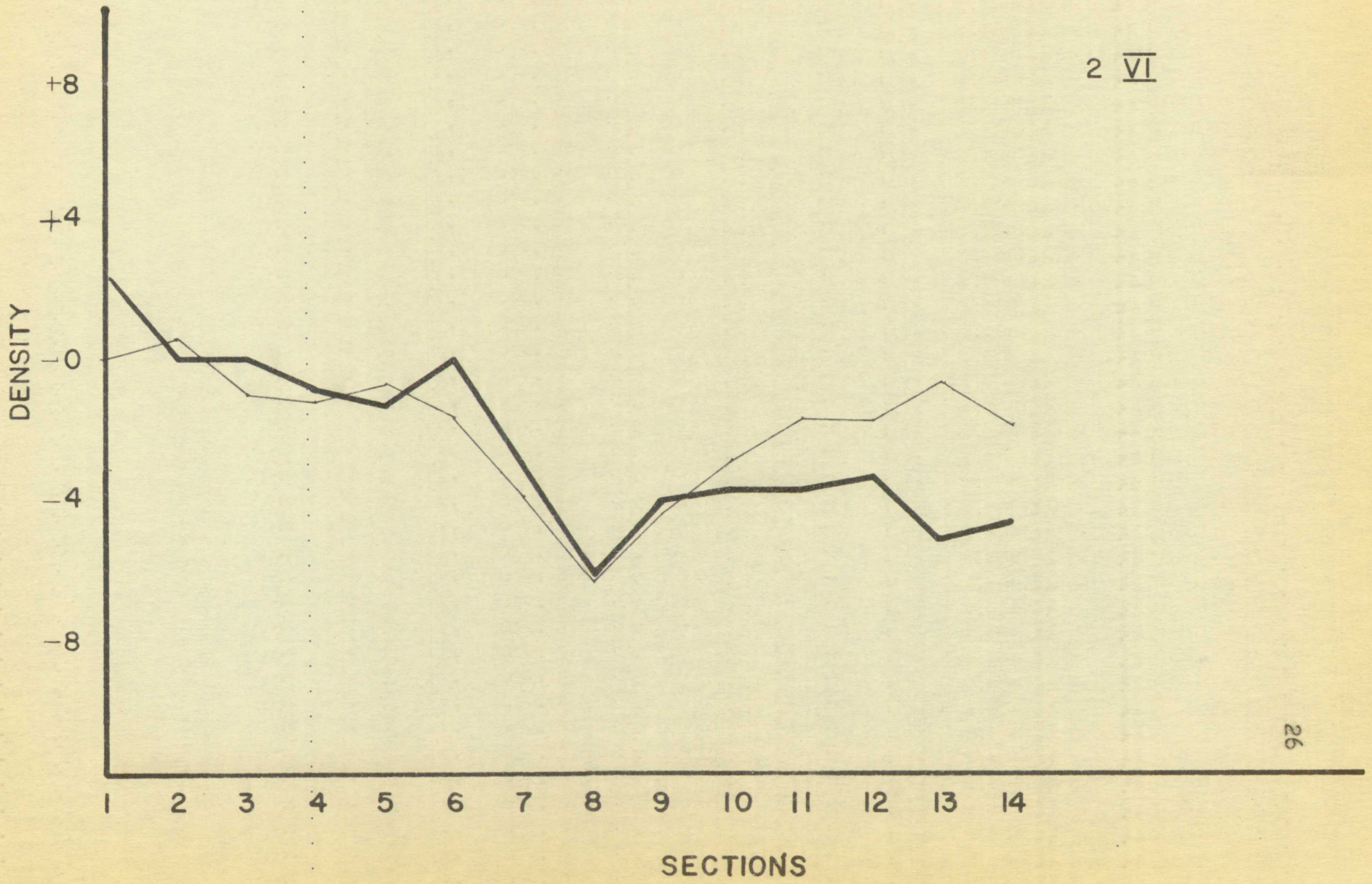
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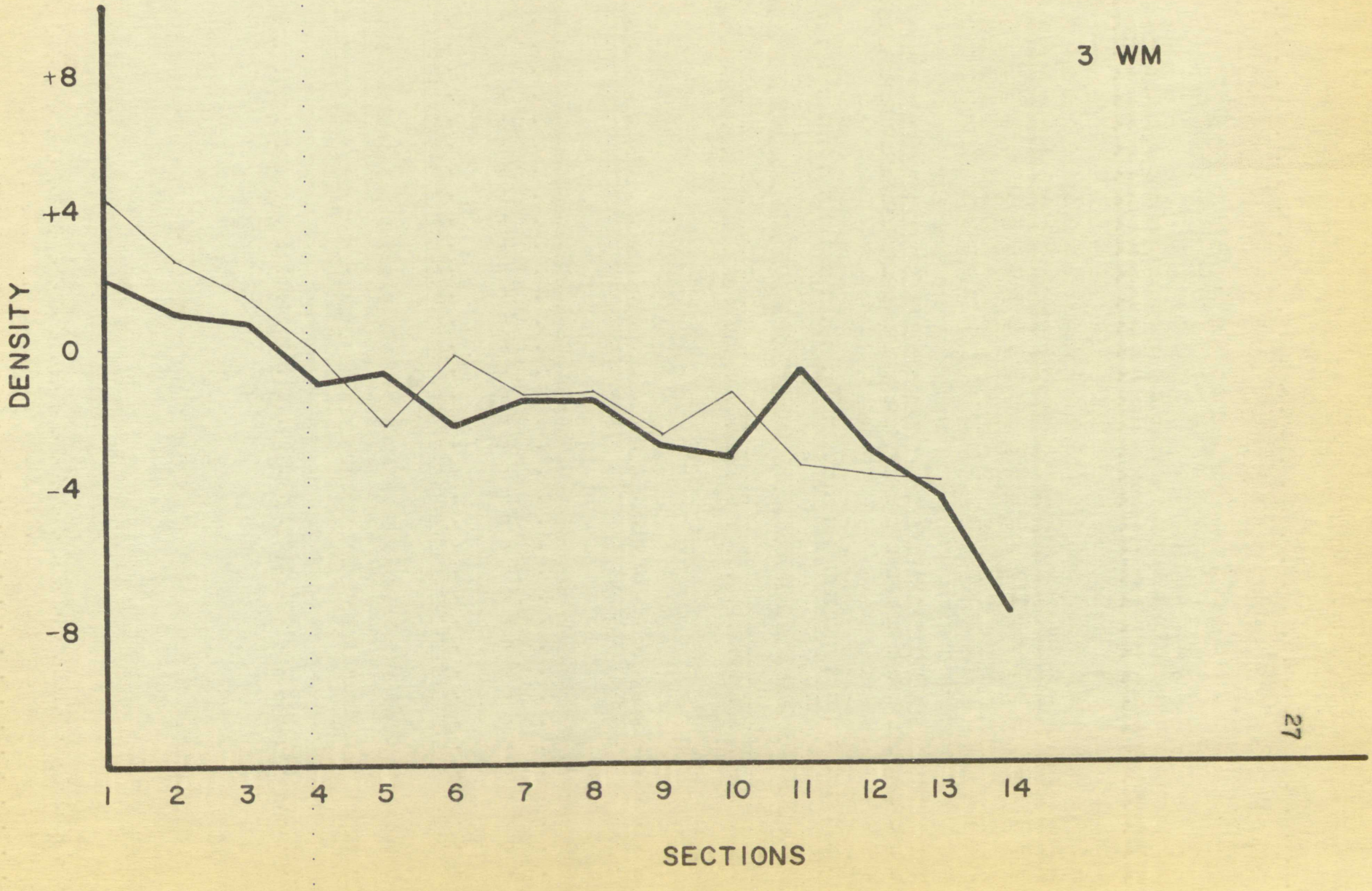
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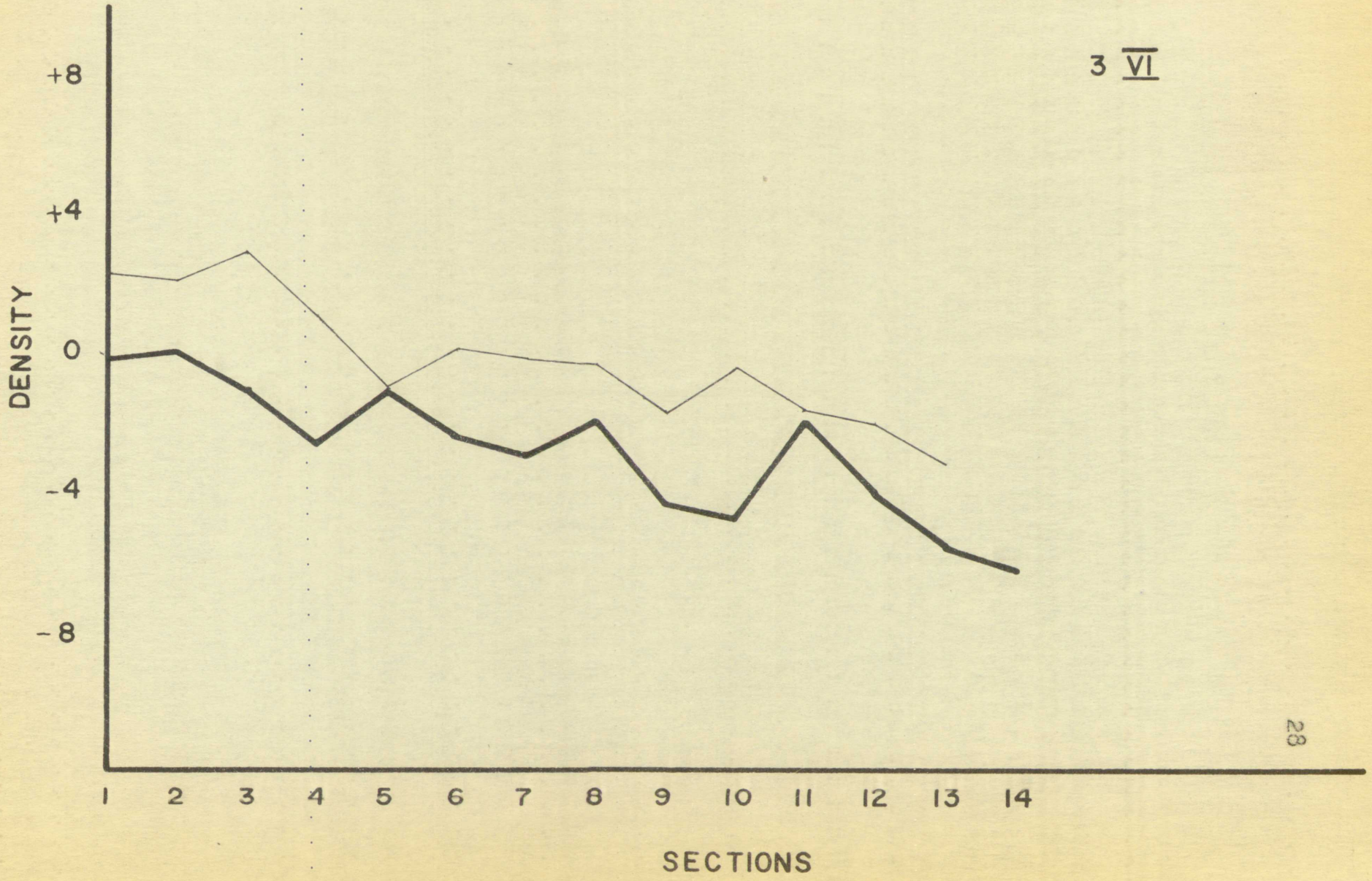
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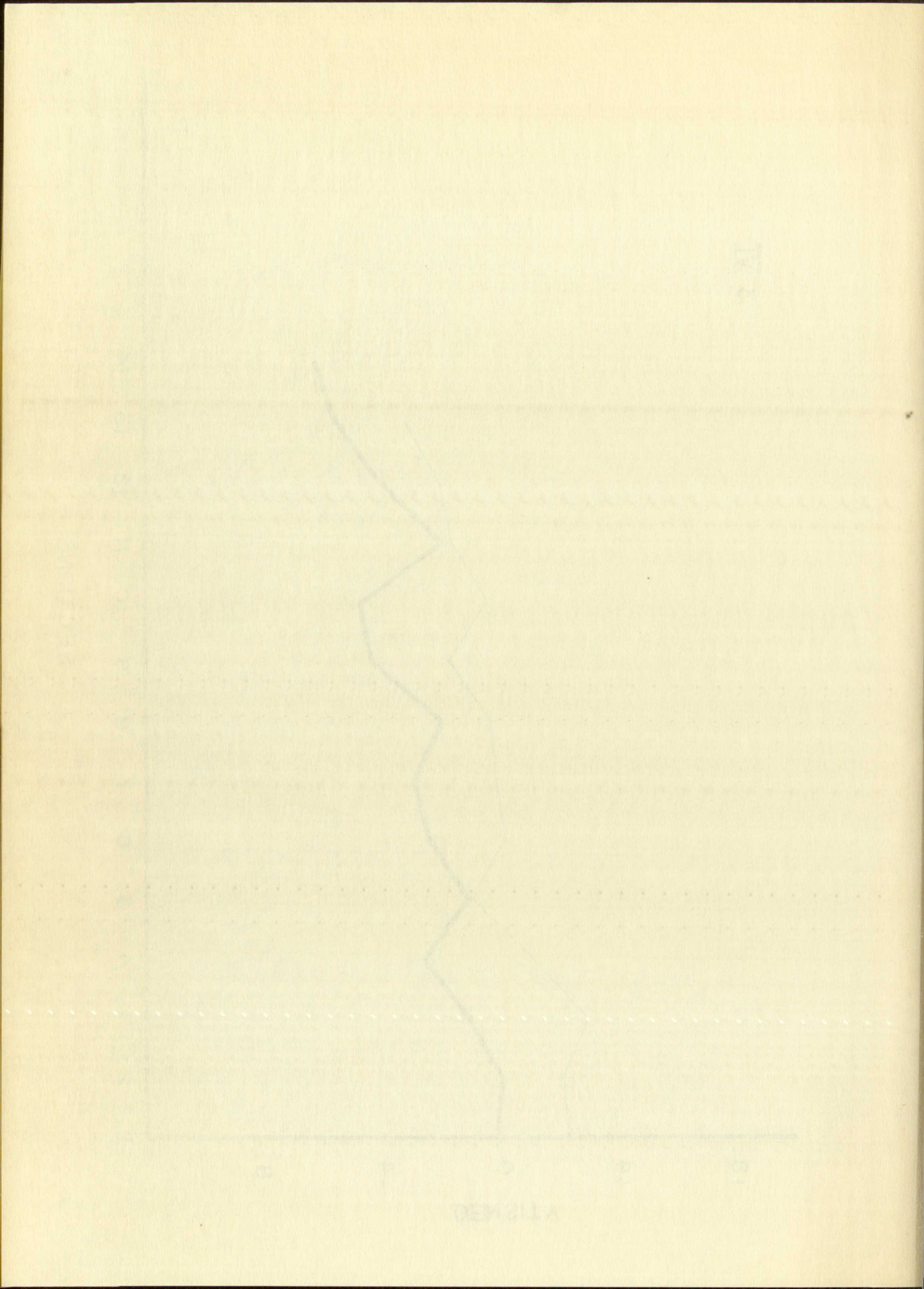

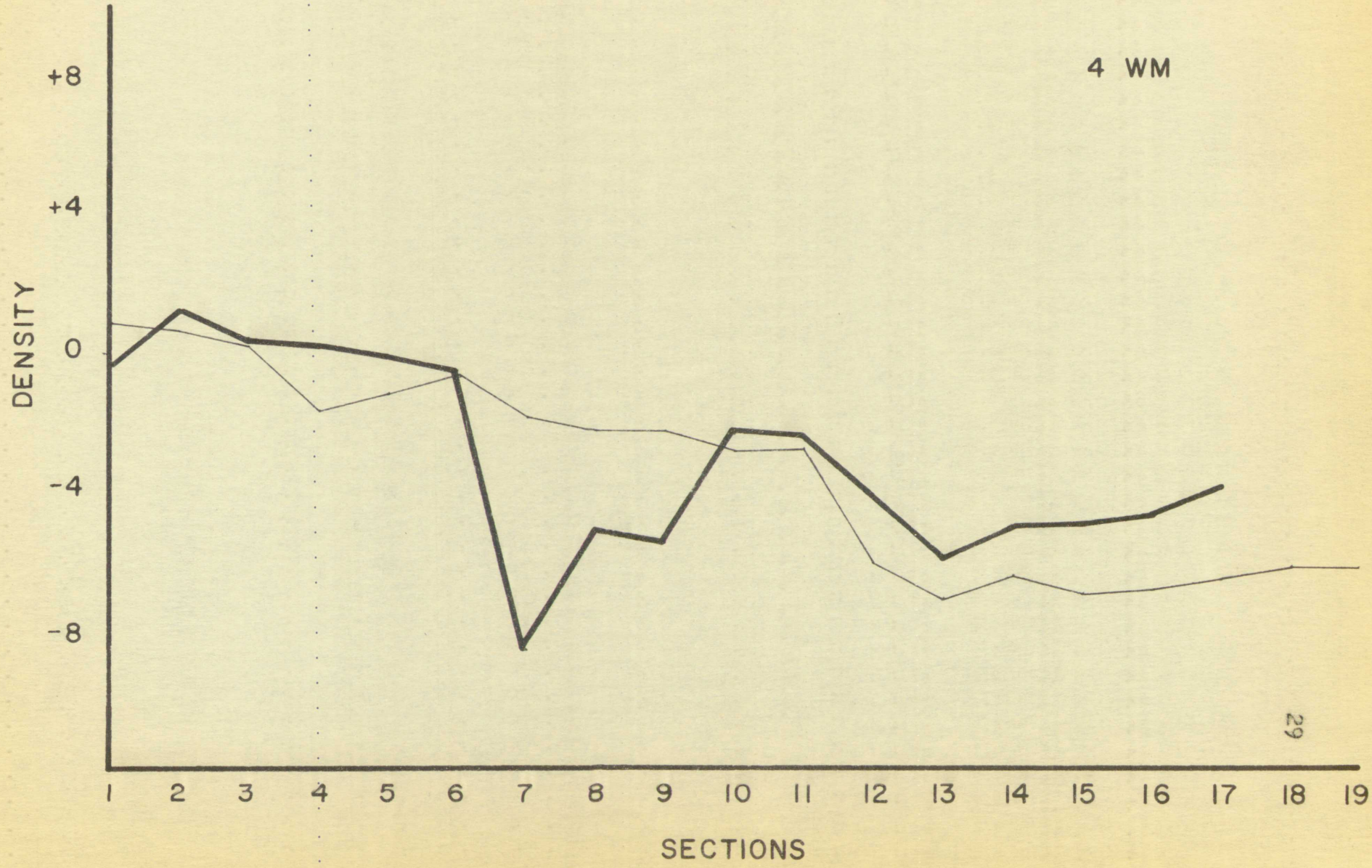
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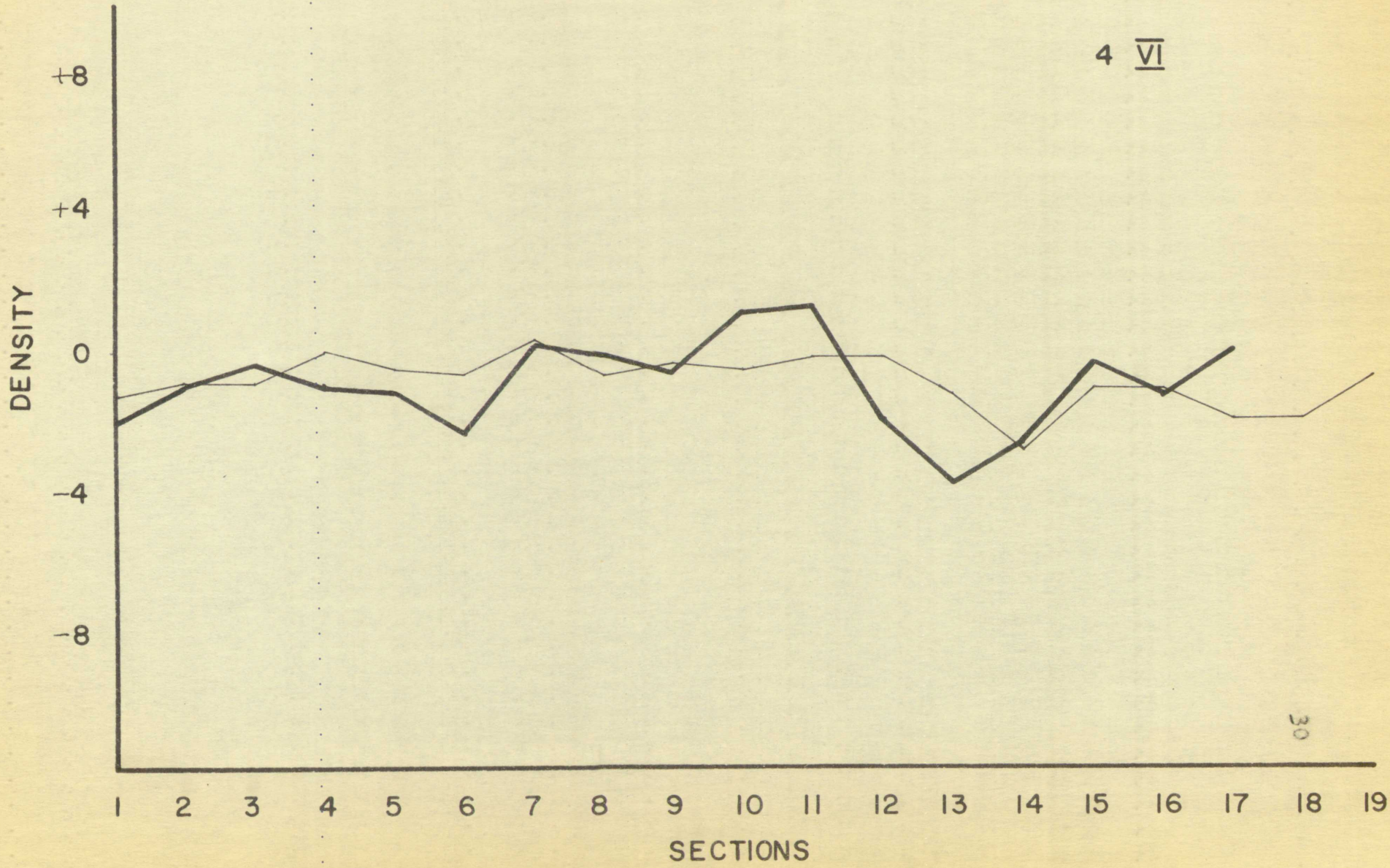
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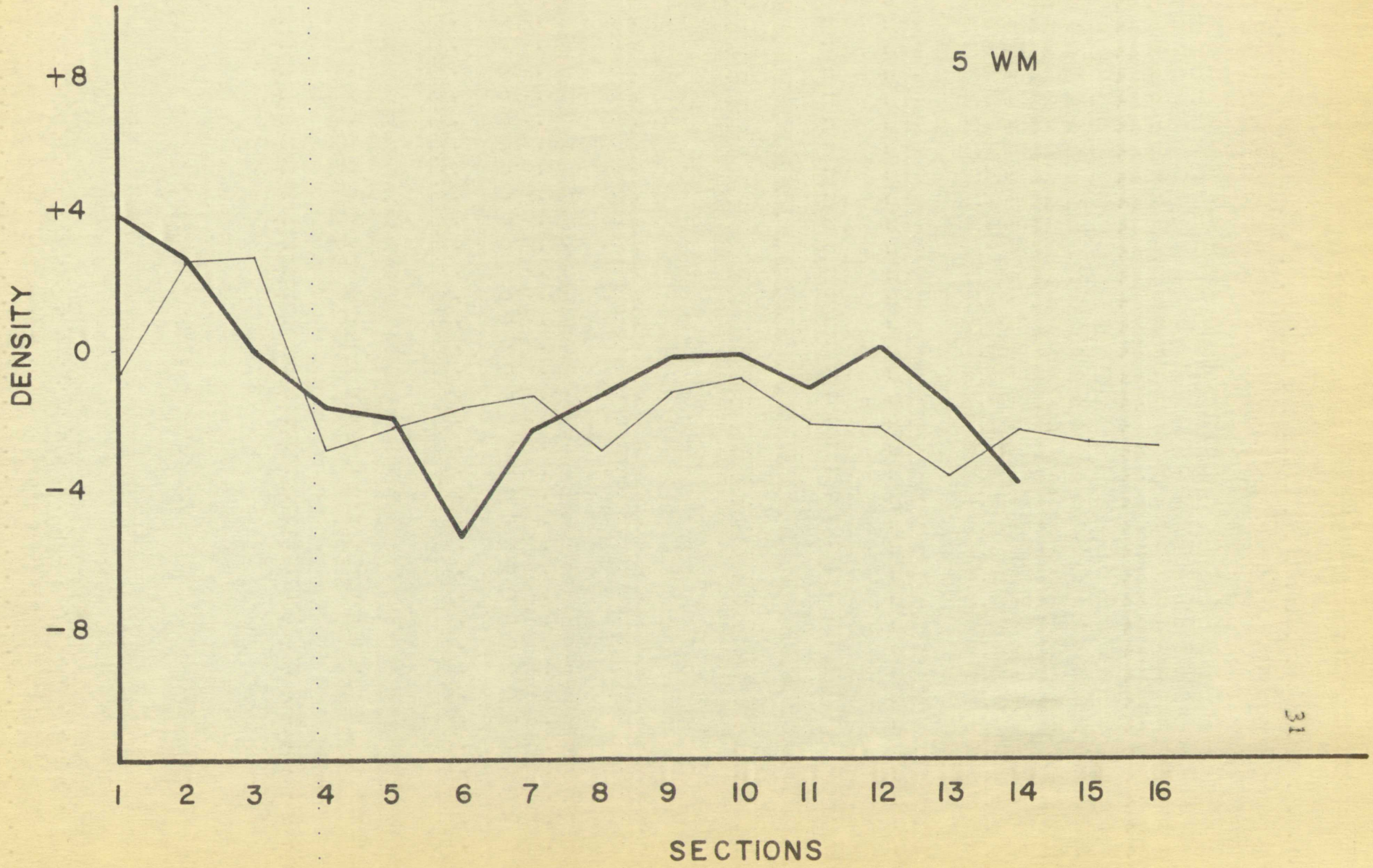
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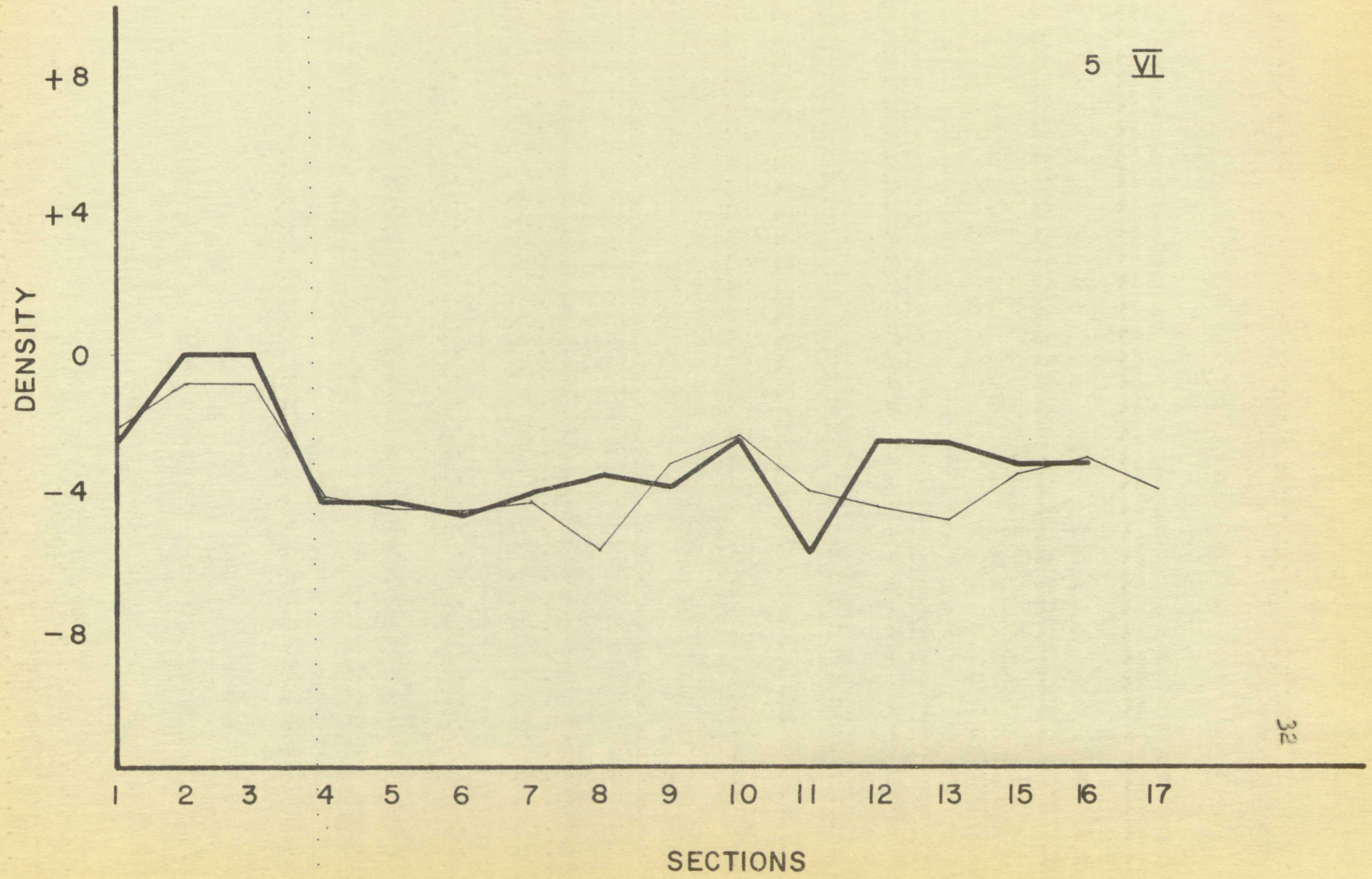
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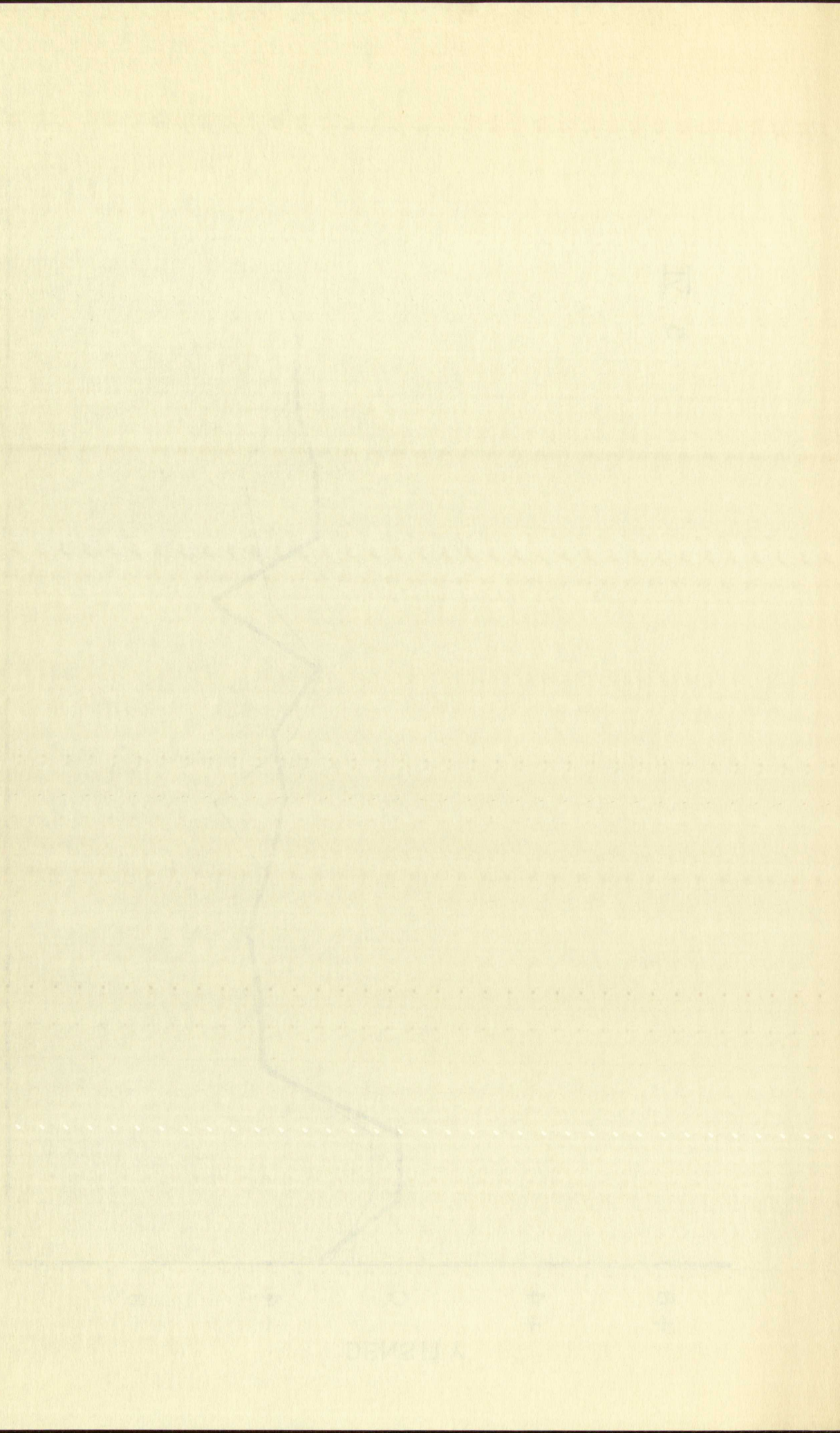

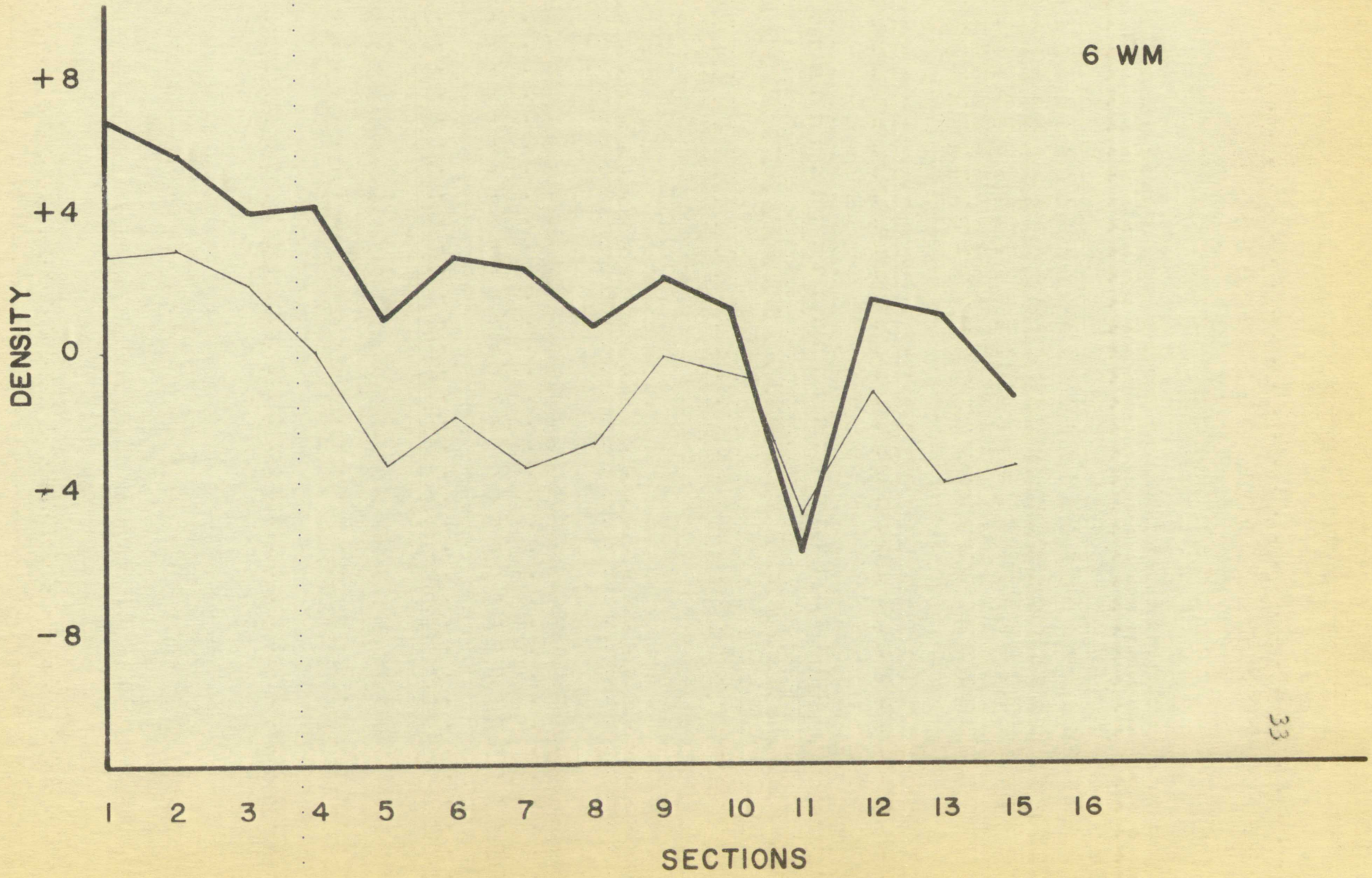
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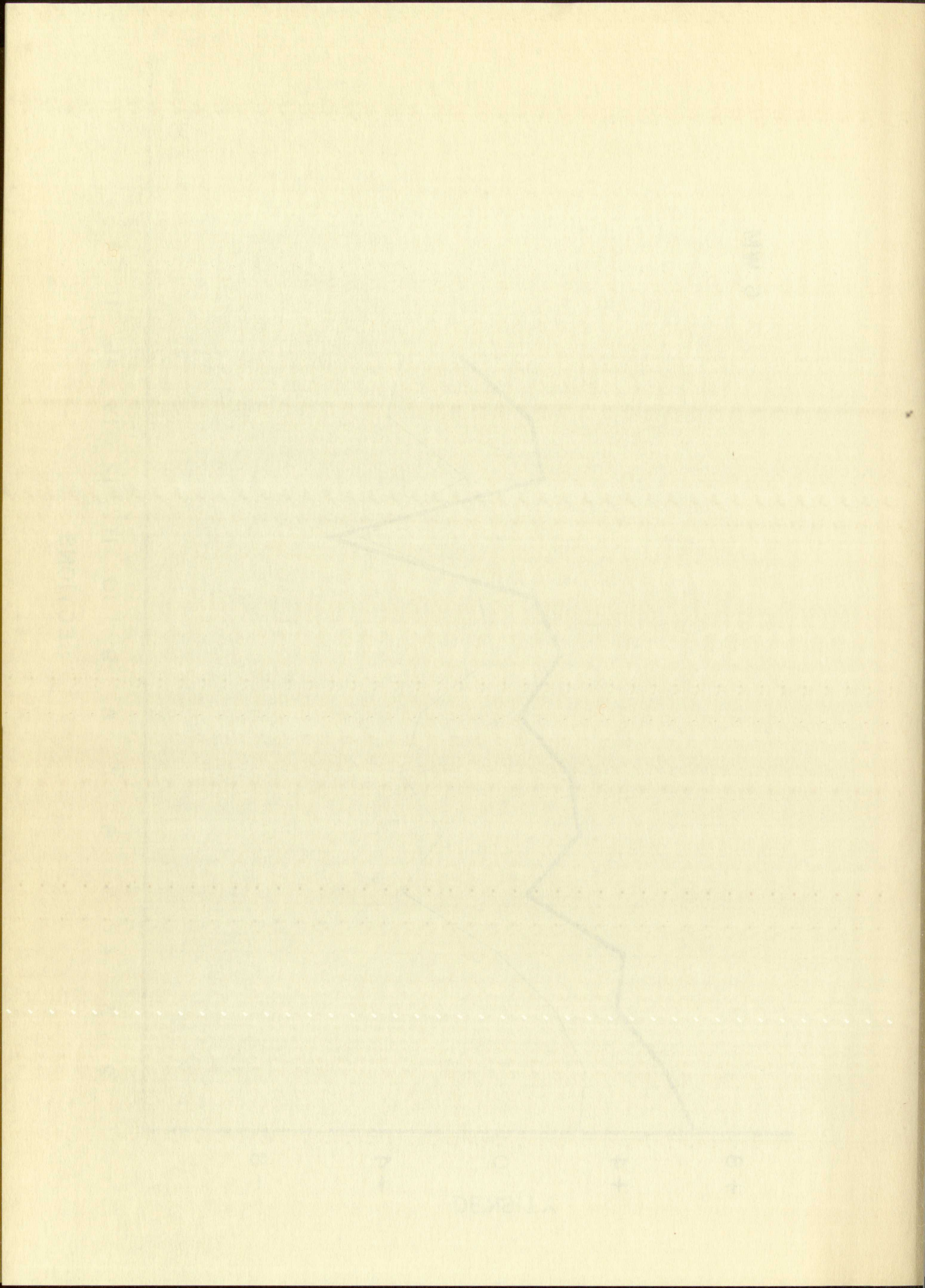

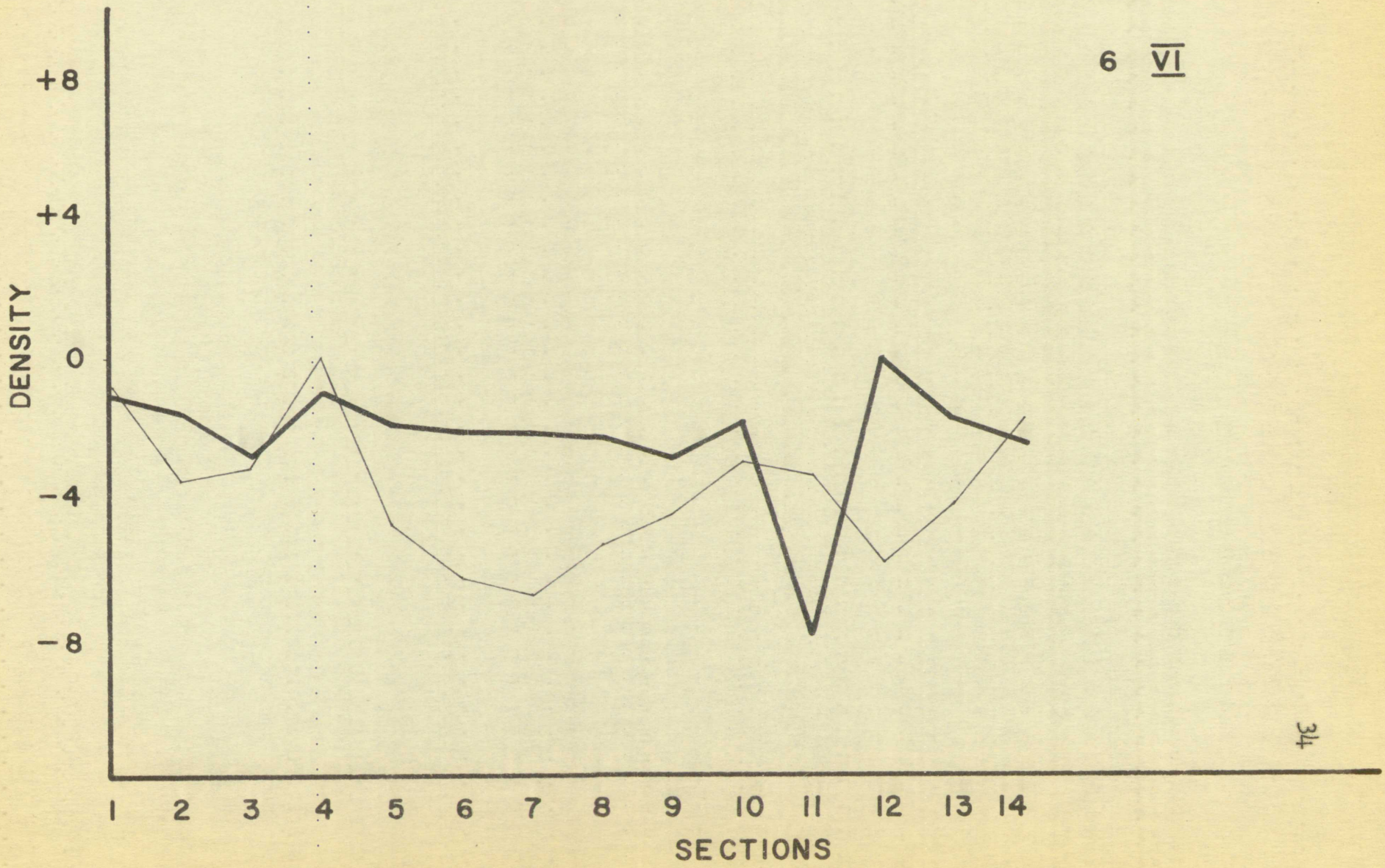
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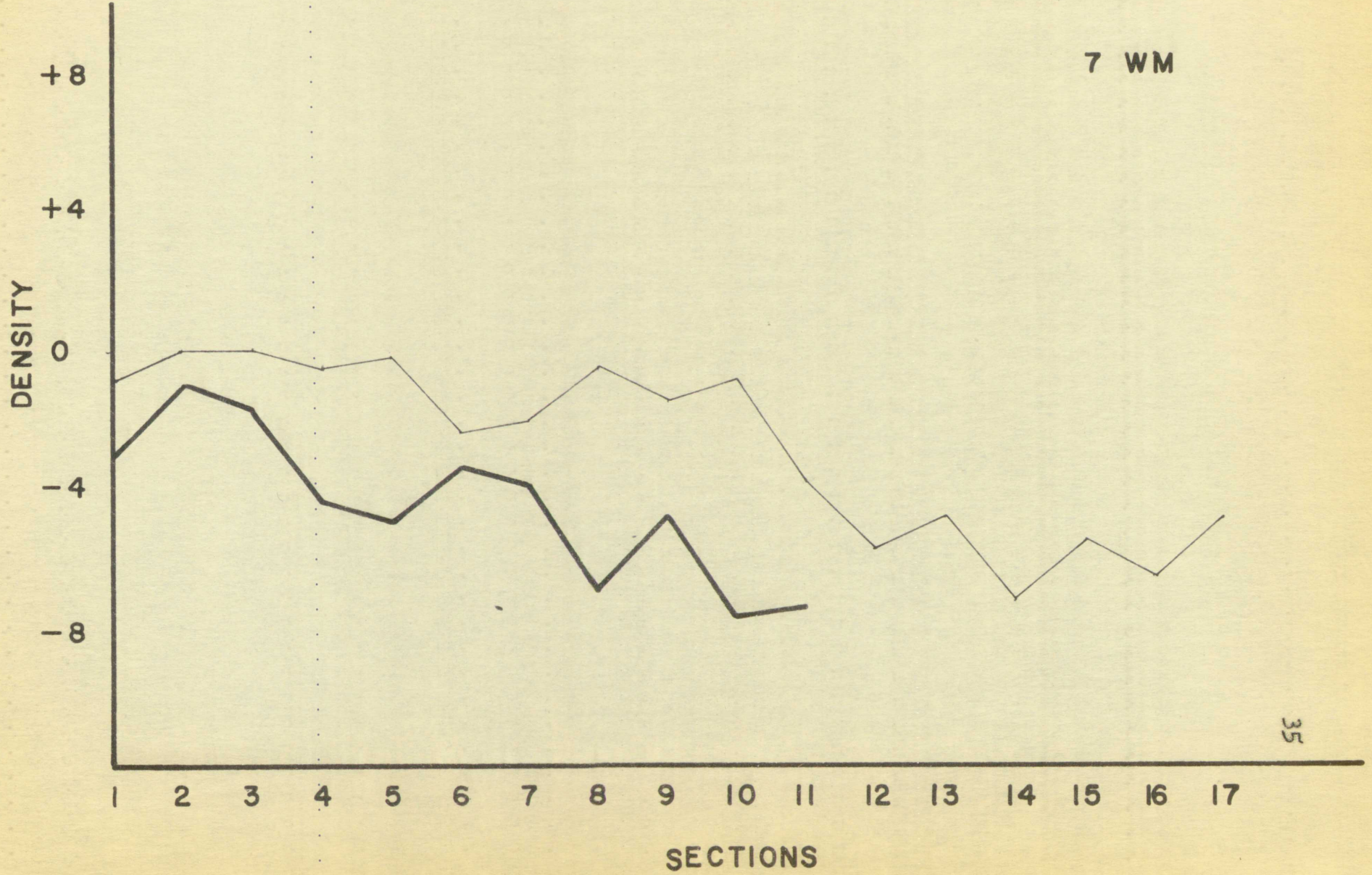
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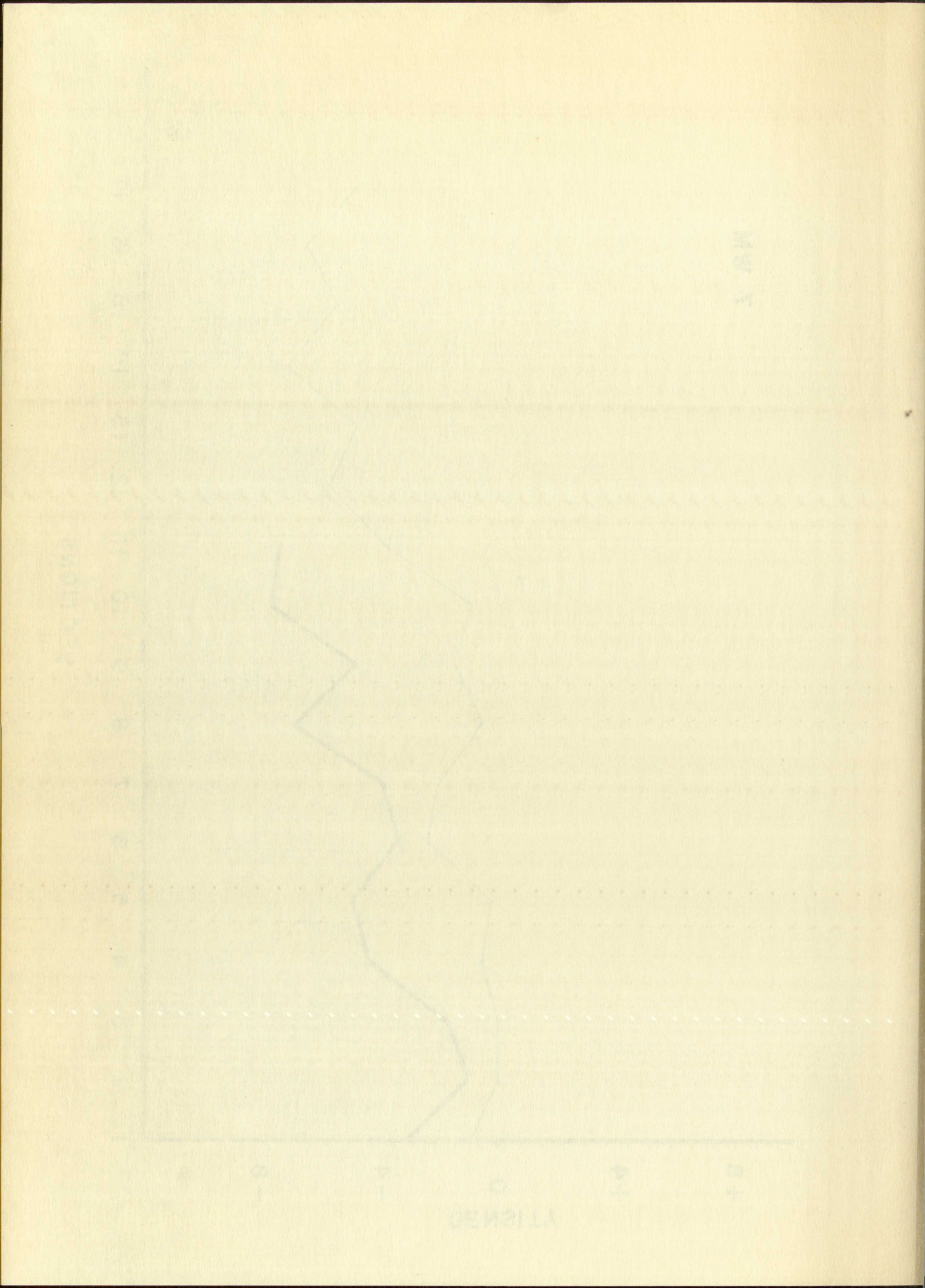

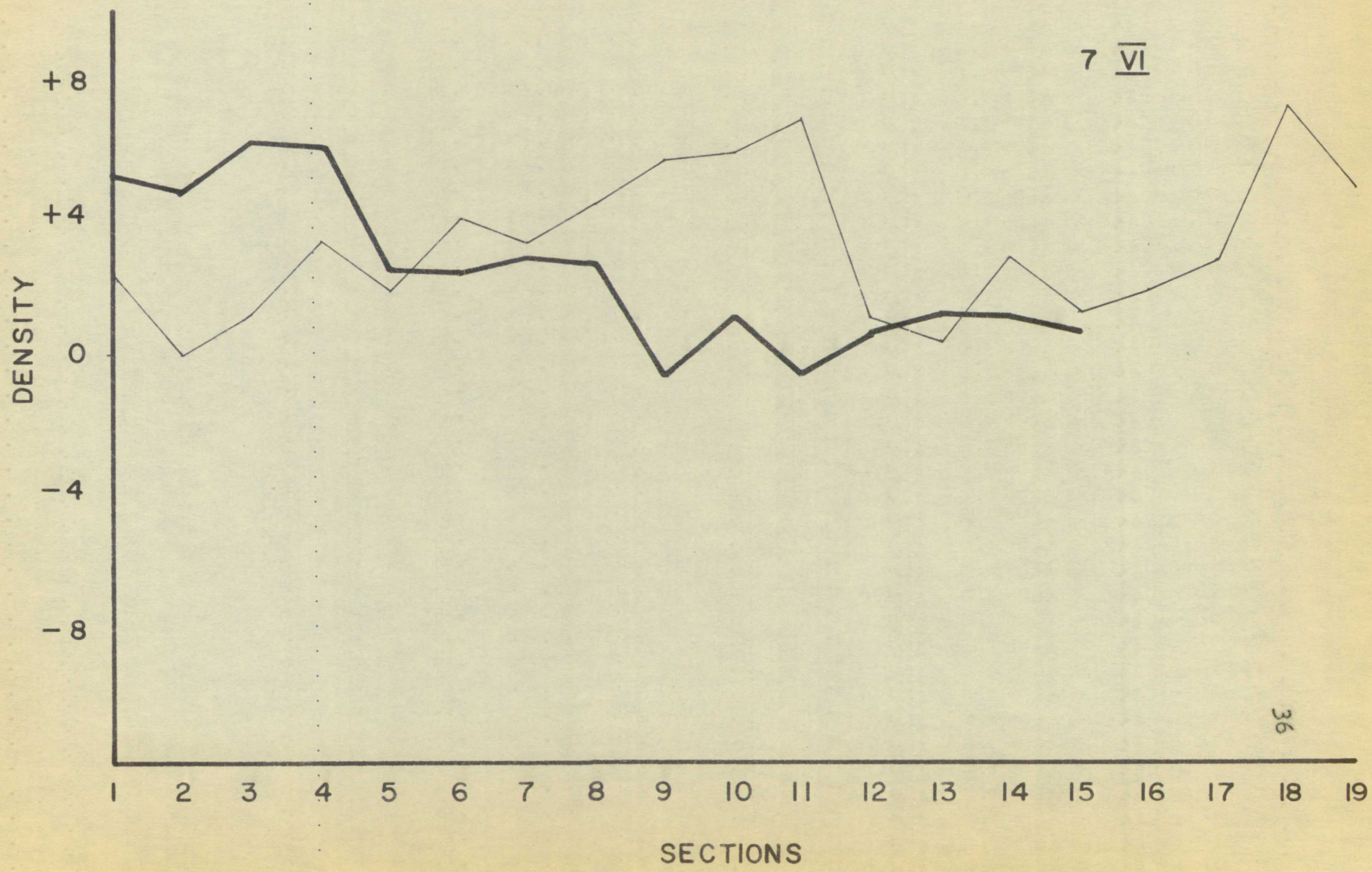
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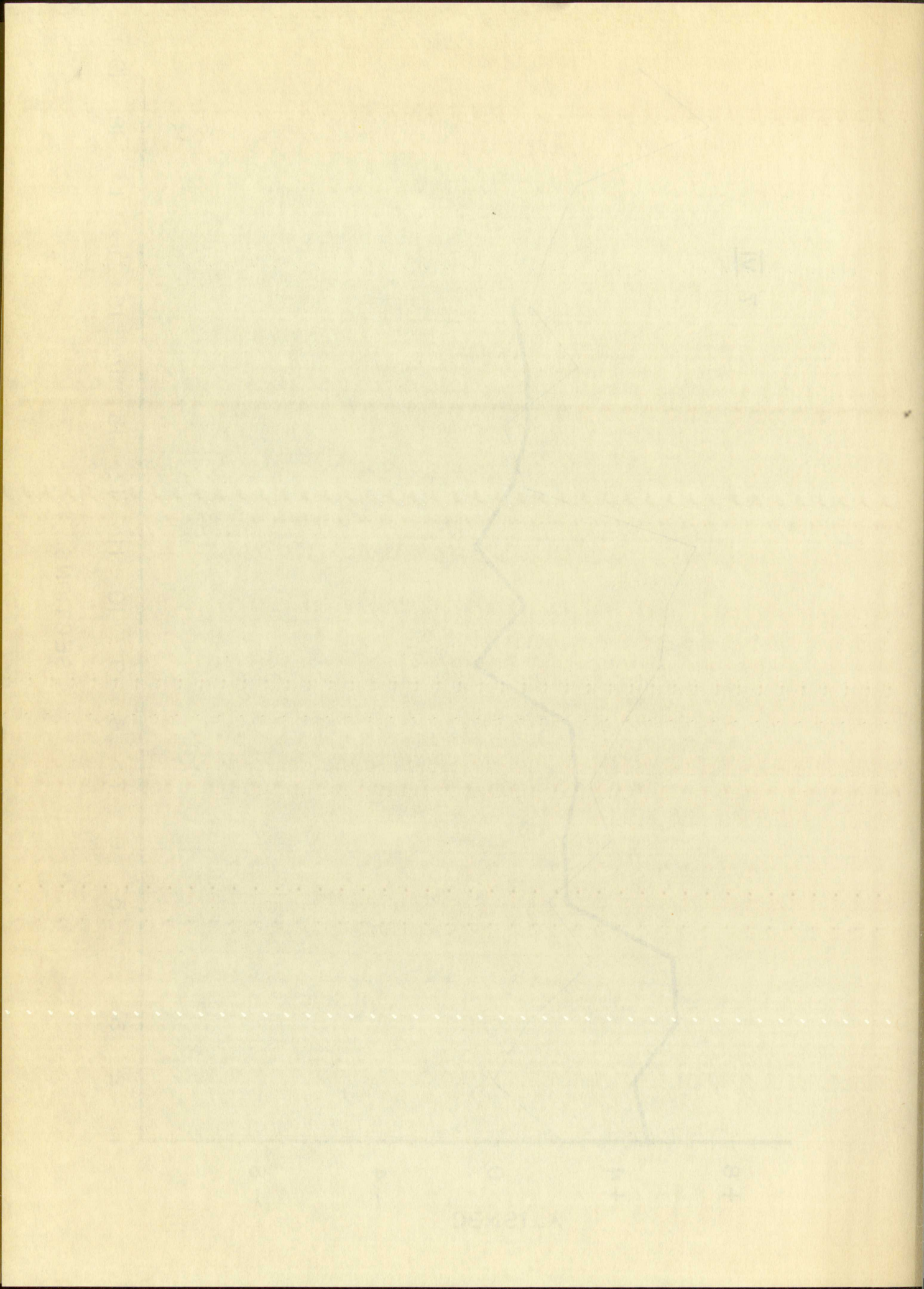

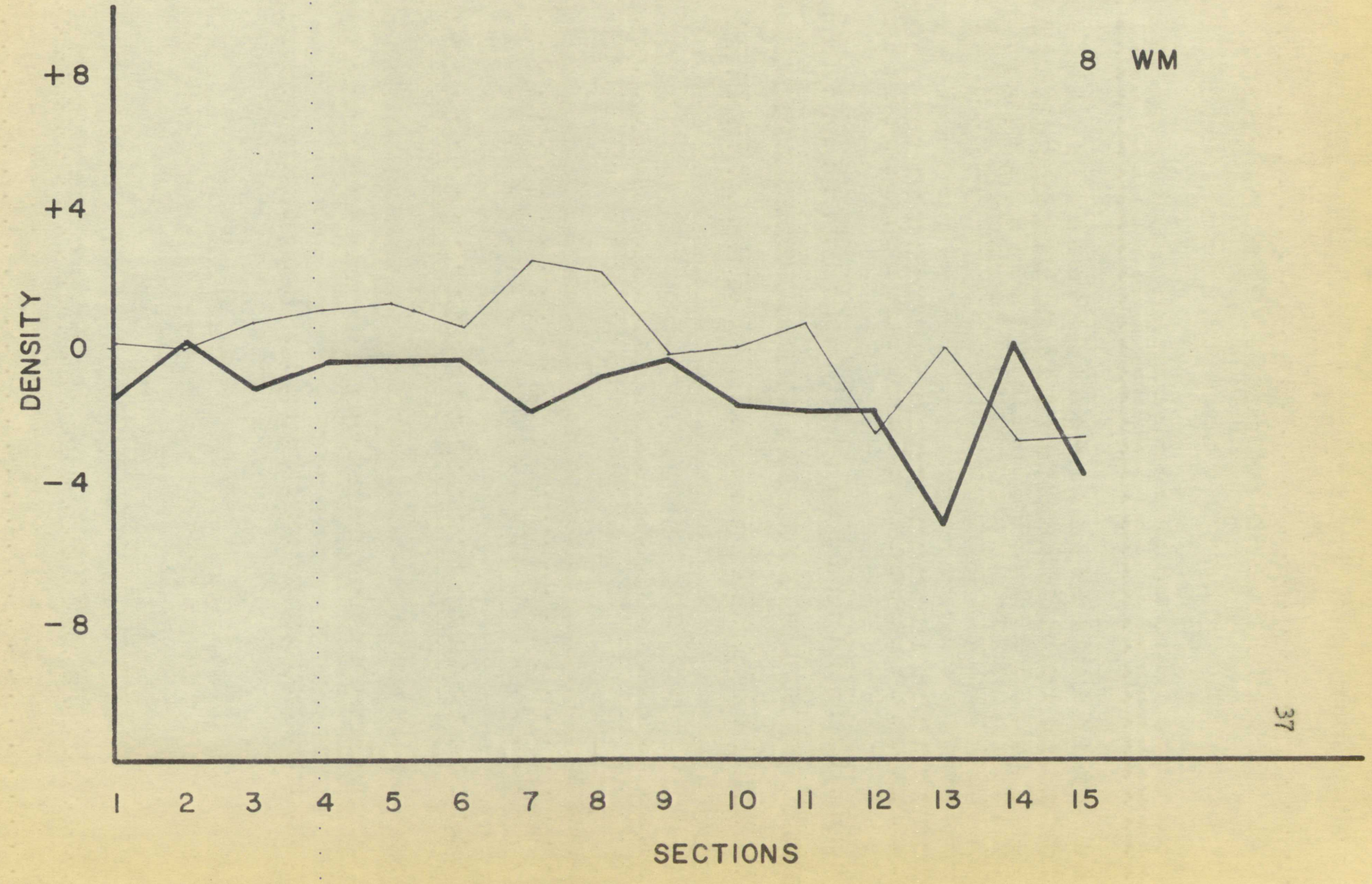
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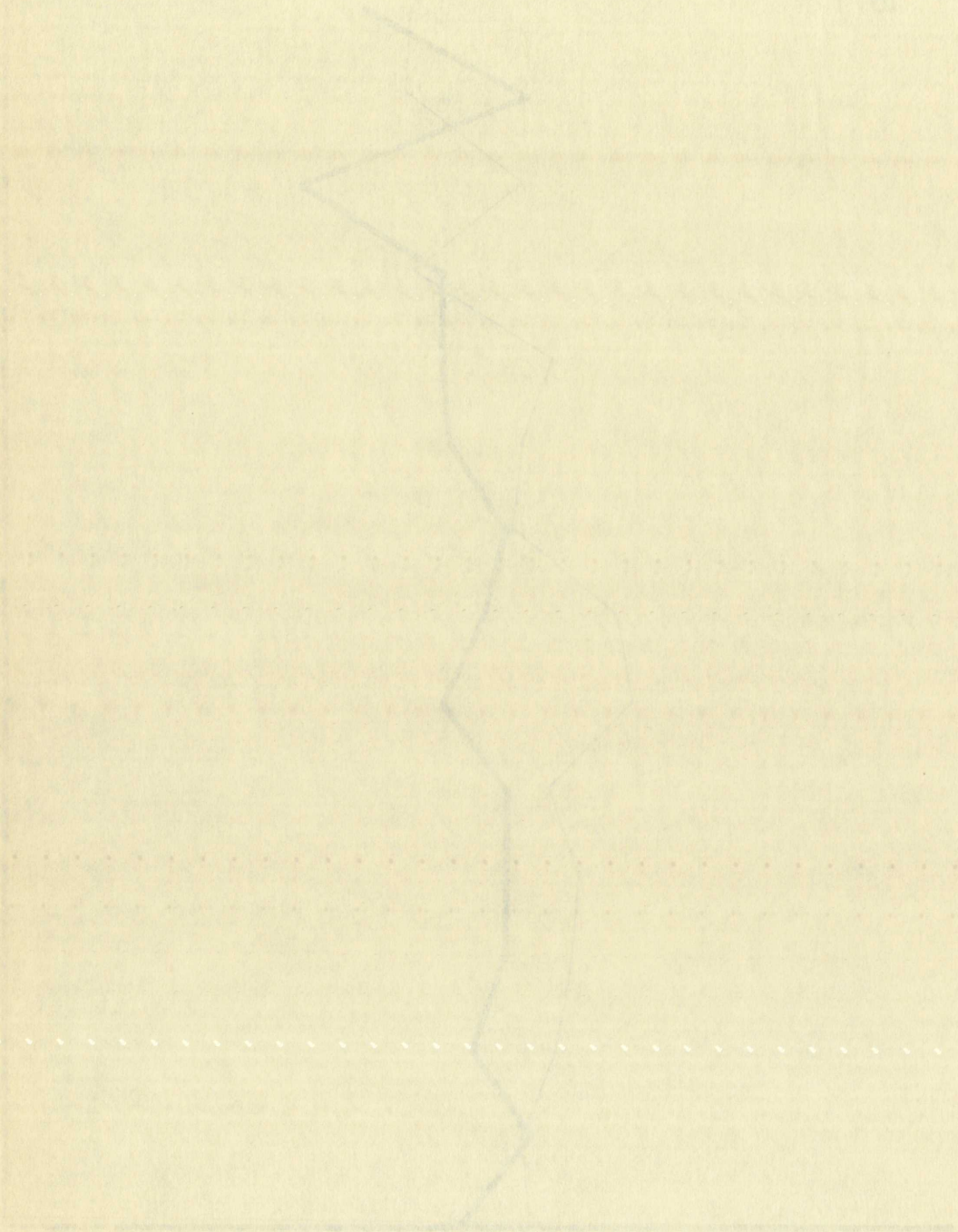

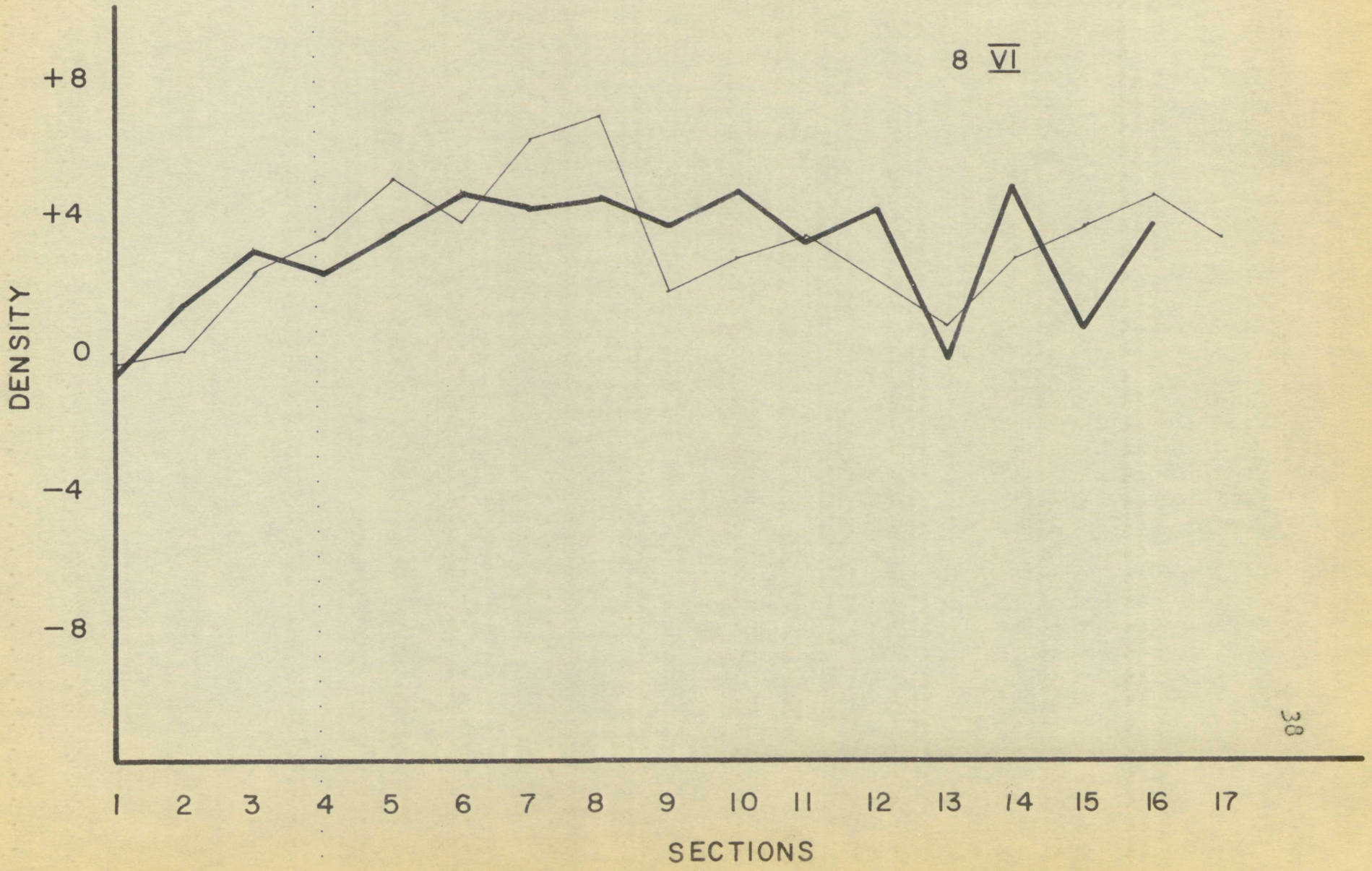
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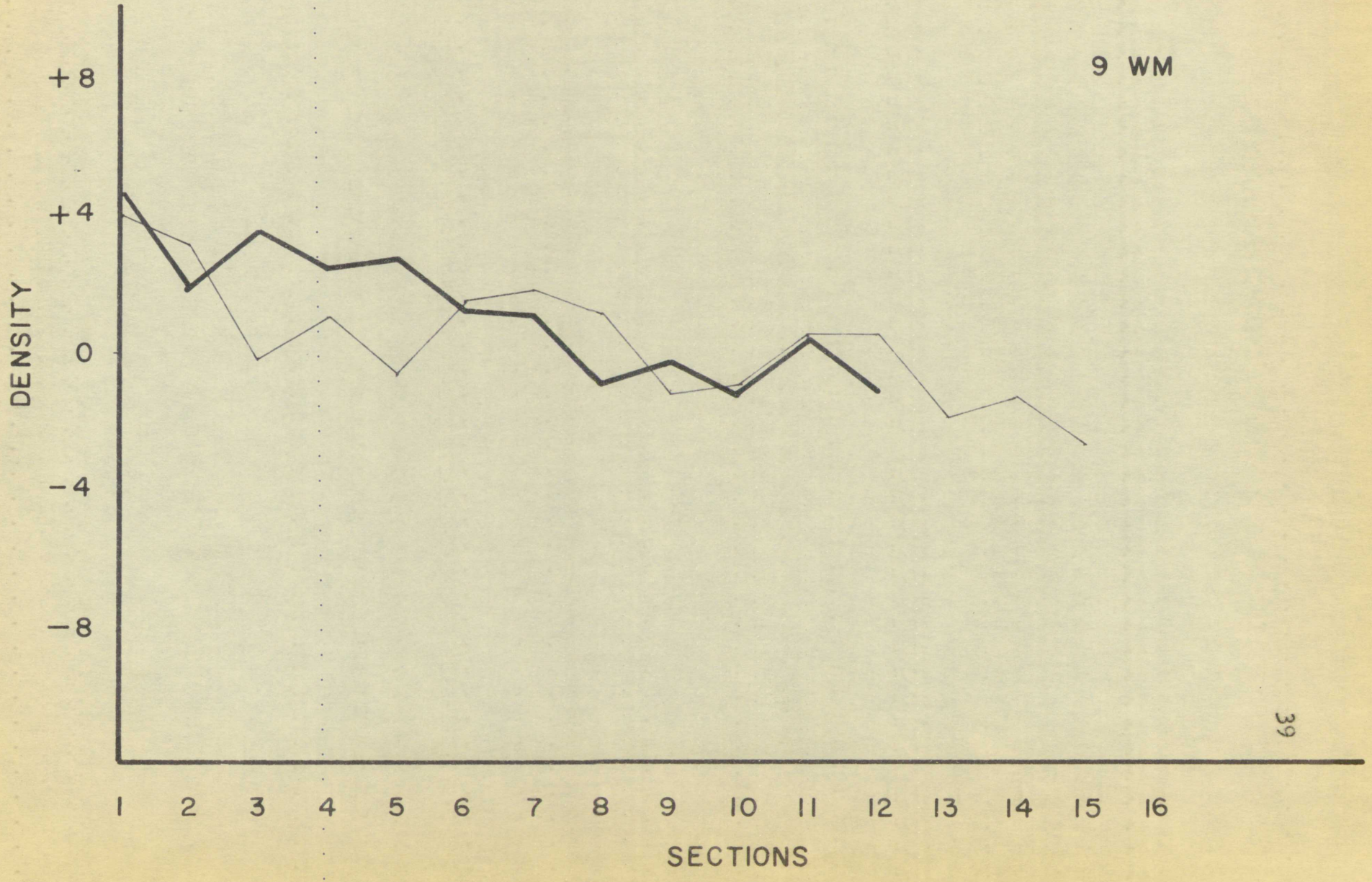
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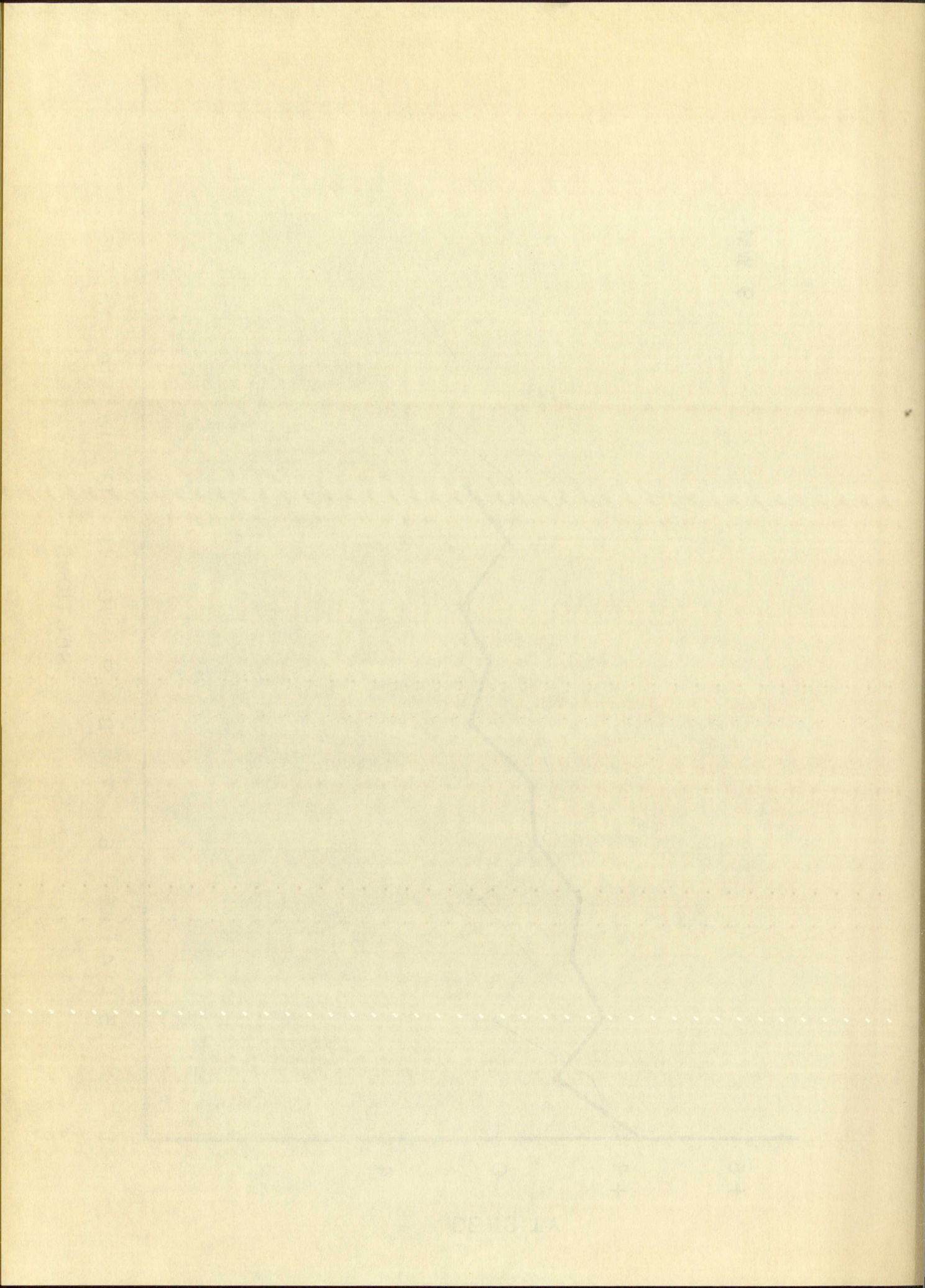

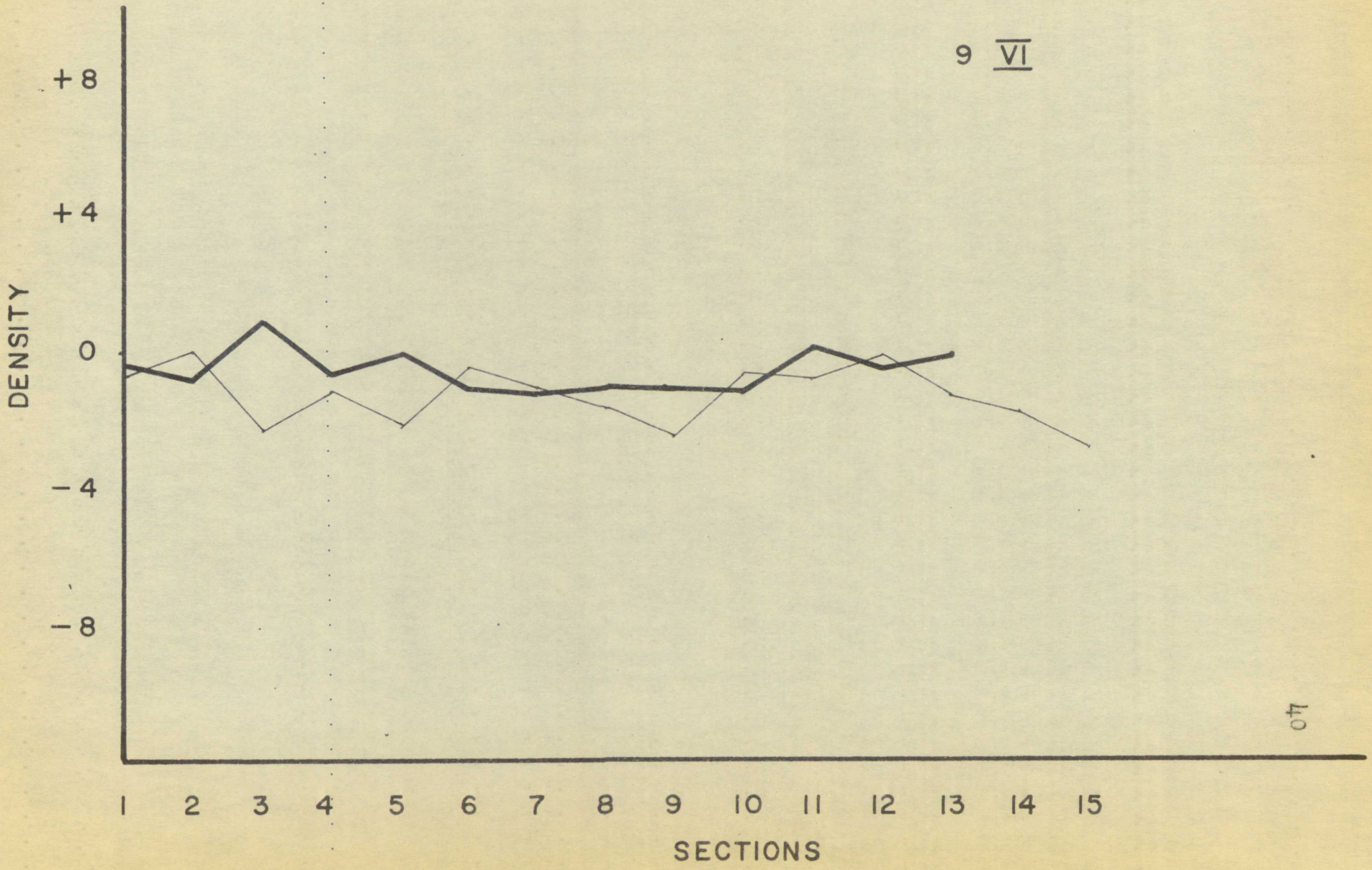
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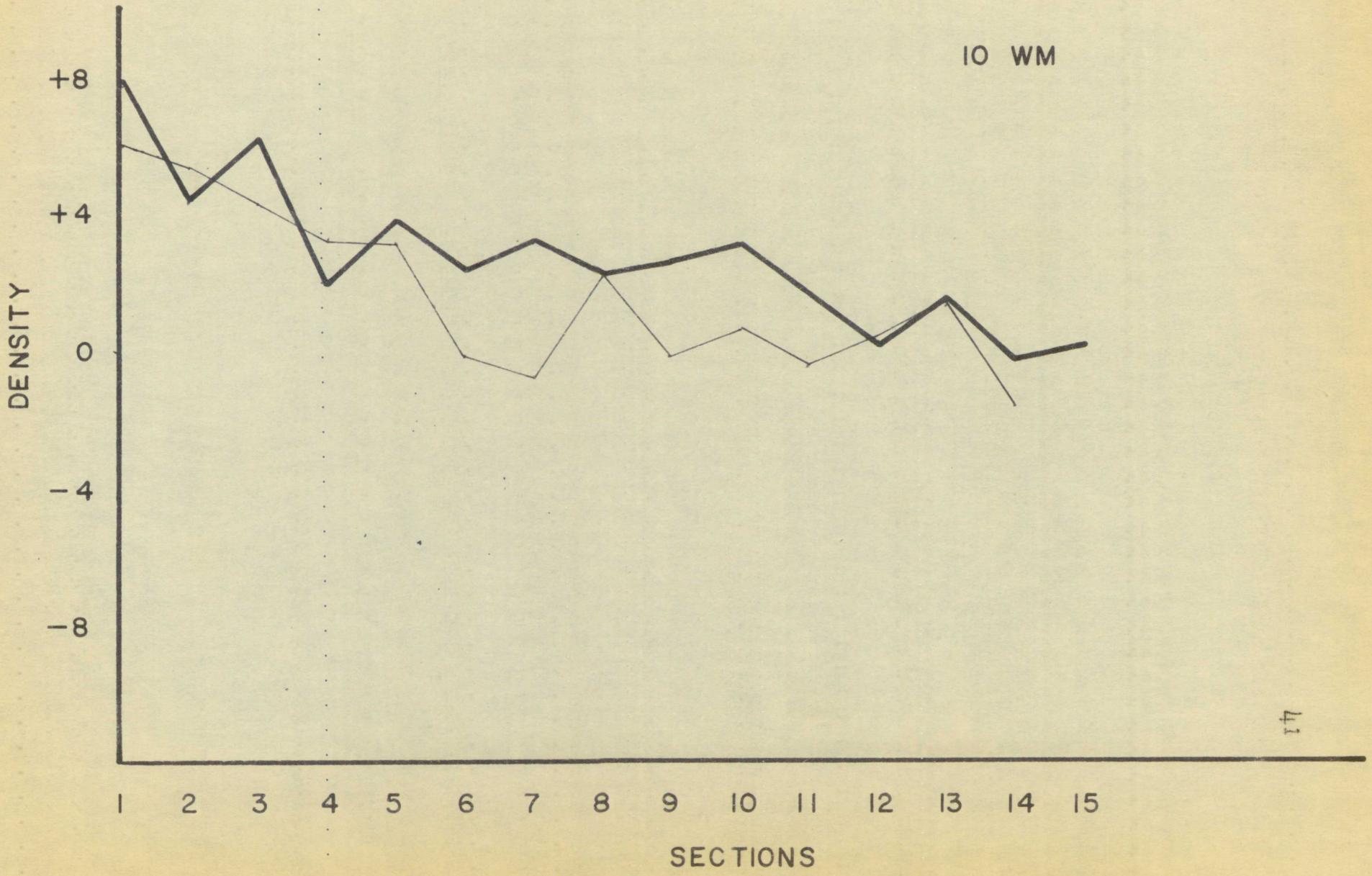
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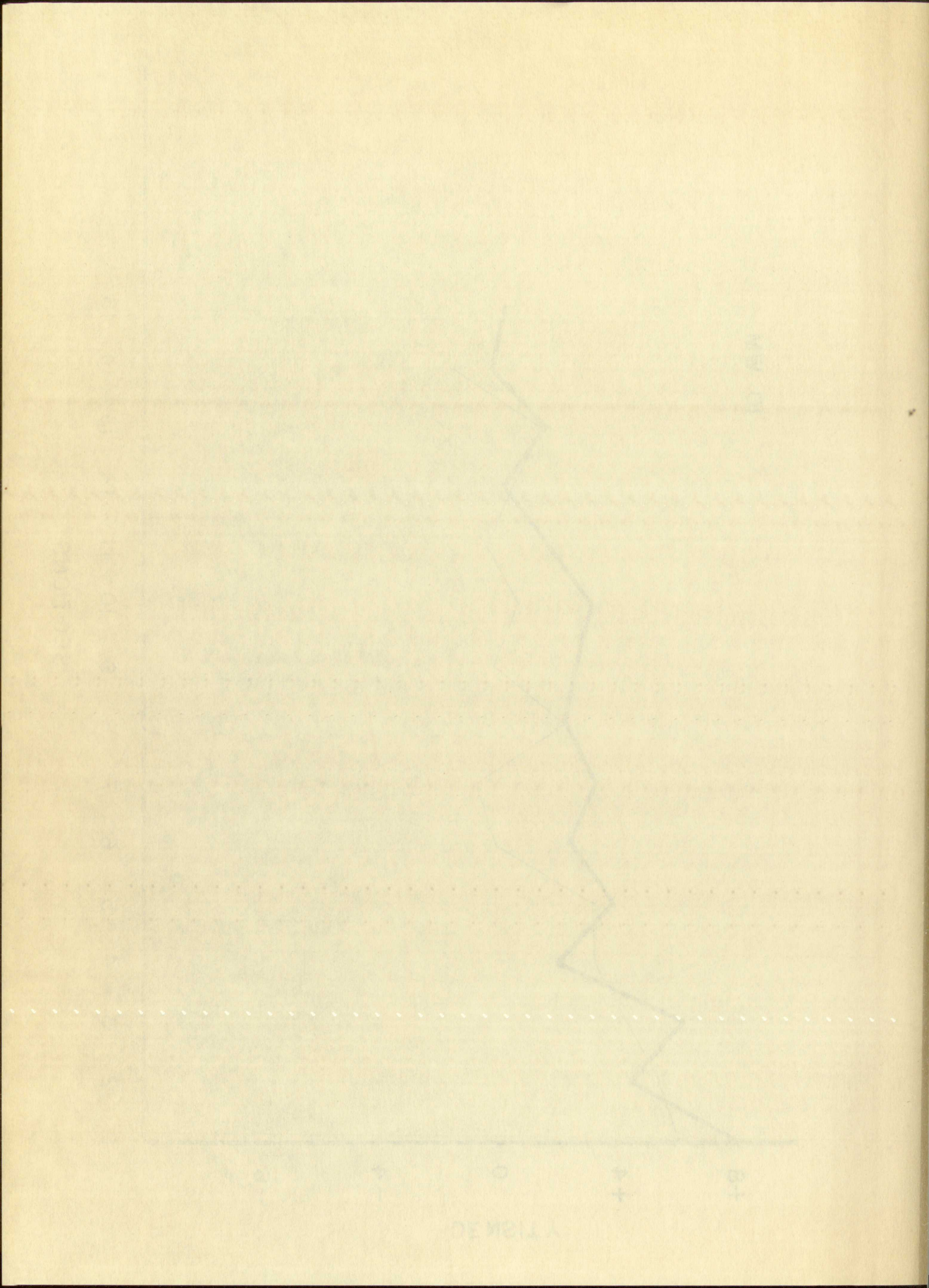

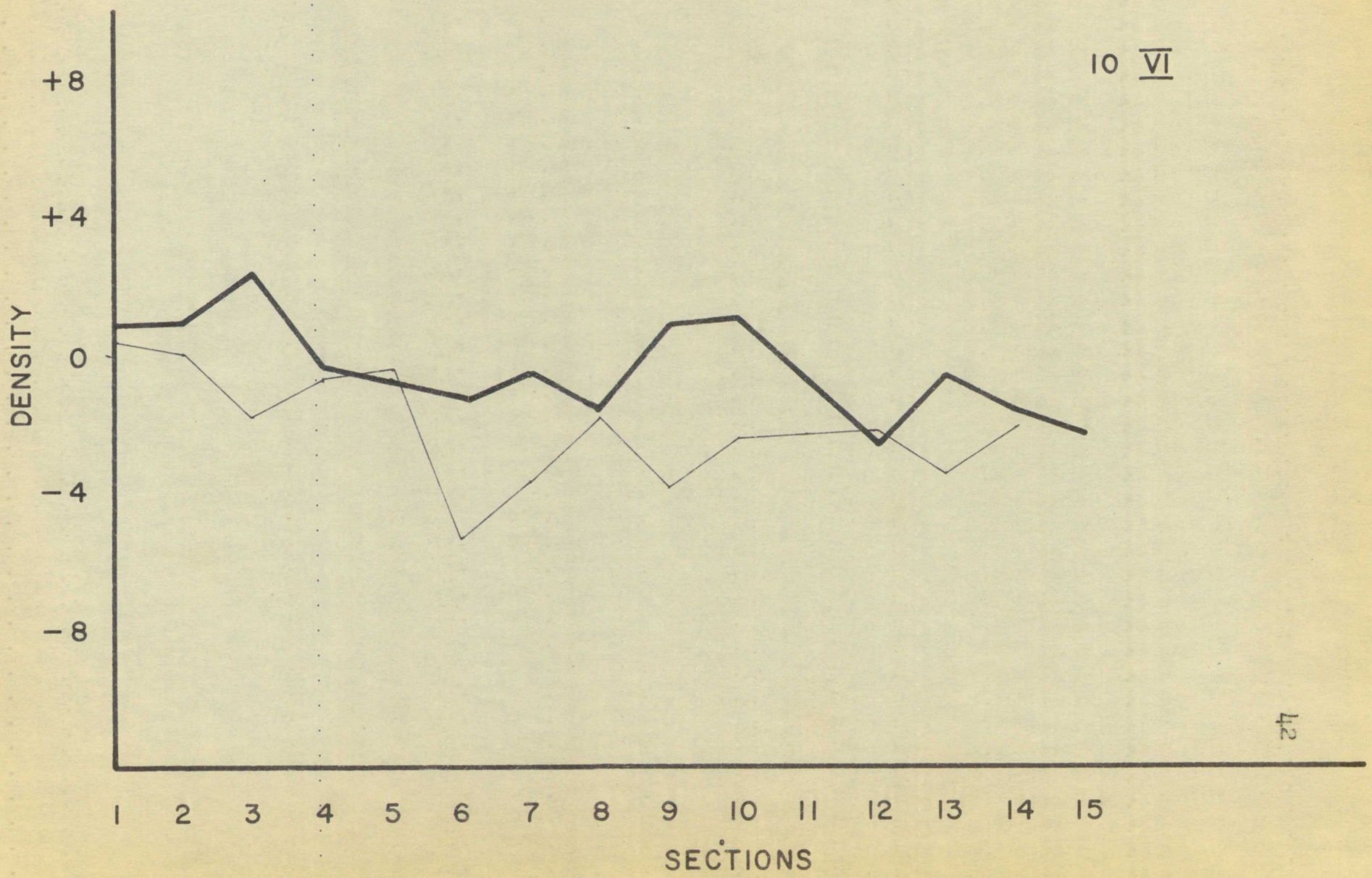
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DISCUSSION

If a relationship between handedness and density of the hemisphere controlling the preferred hand exists, then one should be able to predict the handedness of the animal from the data. Several pilot cases led to the construction of a hypothesis that involved this relationship and specifically stated that the hemisphere controlling the preferred hand would be denser. Consequently, by observation of the graph for any particular animal, one should be able to determine the handedness of this animal. This hypothesis sets up a choice between two hemispheres in each animal, and each of the ten animals is an individual choice situation. There are then ten choice situations, and obviously if prediction of handedness is the key factor in the evaluation of the hypothesis, then the question of chance and its effect upon the results becomes important.

Edwards (1950) cites an experimental design that is quite similar to this problem. Where there are ten sets of items, in each set one being correct and one incorrect, the probability of obtaining all ten sets correctly by chance alone is $\frac{1}{2}^{10}$ or 1 out of 1024. The probability of having nine sets out of ten is 10/1,024, and two incorrect choices is .055 or slightly below the usual criterion of the 5% level.

Inspection of tables II and III indicates that in the case of layer VI, seven out of ten cases could be predicted; and in the white matter only three cases out of ten could be predicted. Statistically, seven out of ten correct choices gives a probability of .17 which does not meet the usual minimum requirement of .05. Even if the hypothesis were reversed, that is to say that the lightest hemisphere had predictive value, the white matter data would also give a P of .17 which is at a chance level. Consequently the hypothesis which states that density of a hemisphere (in the white matter and layer VI as measured in this experiment) has predictive value in relation to handedness is not supported.

After this general negative result, the graphs were examined in terms of a second hypothesis, namely that the pattern of the graphs might be related to handedness. This approach also turns out to be unfruitful. Many pattern hypotheses were developed in terms of results on one or two graphs, but broke down when subjected to more thorough examination.

Actually, in some of the cases, the graphs could not be classified by inspection and it was necessary to compute means, obviously not significant, in order to classify for density.

Table II -- Predictive value of WM data

Rat No.	Dense Hemisphere (WM)	Preferred Hand	Prediction
1	R	R	-
2	L	R	+
3	L	R	+
4	R	R	-
5	R	R	-
6	L	L	-
7	L	L	-
8	R	L	+
9	L	L	-
10	L	L	-

3/10 correct

Table III -- Predictive value of layer VI data

Rat No.	Dense Hemisphere (VI)	Preferred Hand	Prediction
1	L	R	+
2	L	R	+
3	L	R	+
4	L	R	+
5	R	R	-
6	L	L	-
7	R	L	+
8	R	L	+
9	R	L	+
10	L	L	-

7/10 correct

Consequently, it can be concluded that this first step in the examination of cortical tissue for structural changes has yielded a negative result. At the same time it should be said that this step is a first but simple approach to the problem. With the present technique two discrete readings have been taken from two arbitrarily selected spots in the cortex. The point is that if all the lamination of the cortex had been examined with this particular stain or perhaps with some other stain the results might not have been negative, and at the present time we have developed a combination of instrumentation that will achieve this goal of laminar examination. The cortex is continuously scanned by the light beam and this information is fed to an oscilloscope. The result is a density lamination curve such as Figure 2.

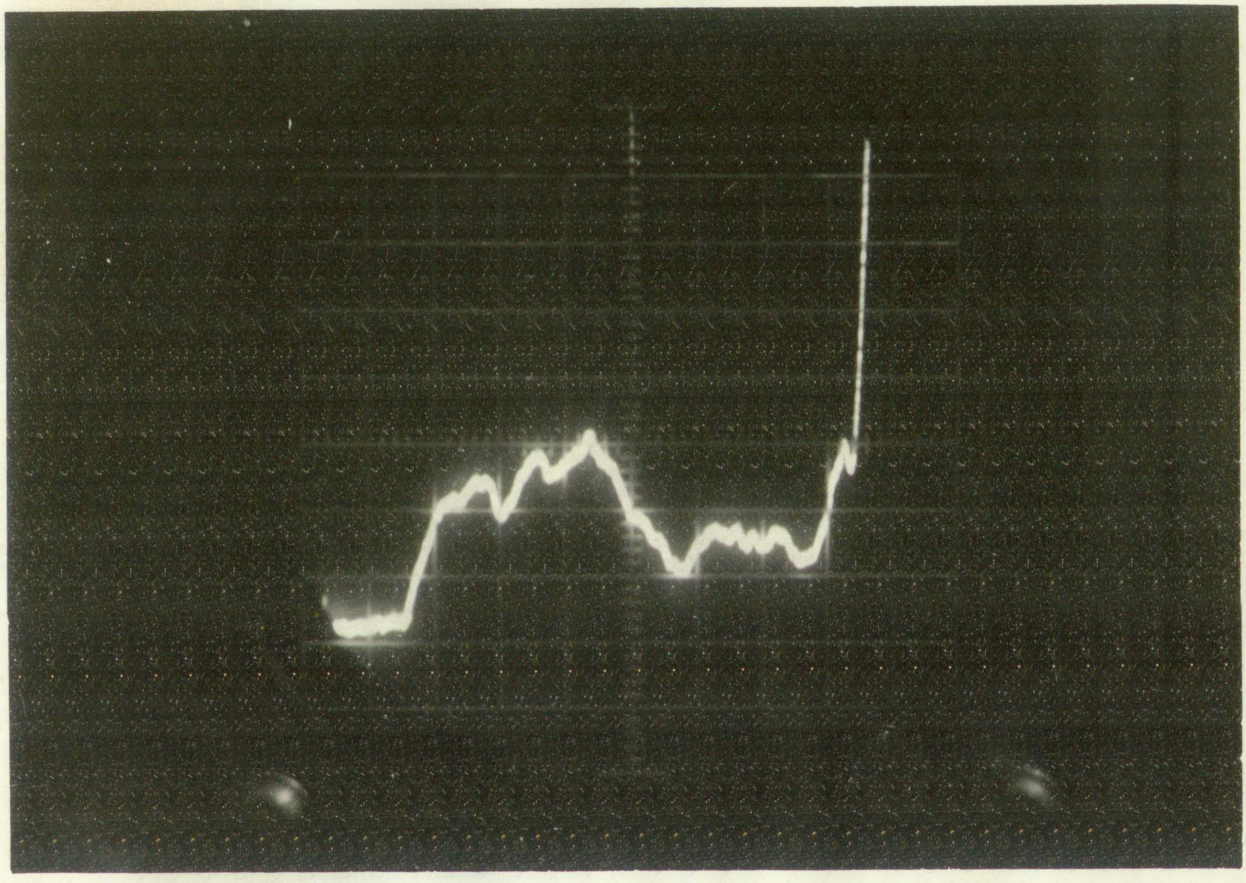
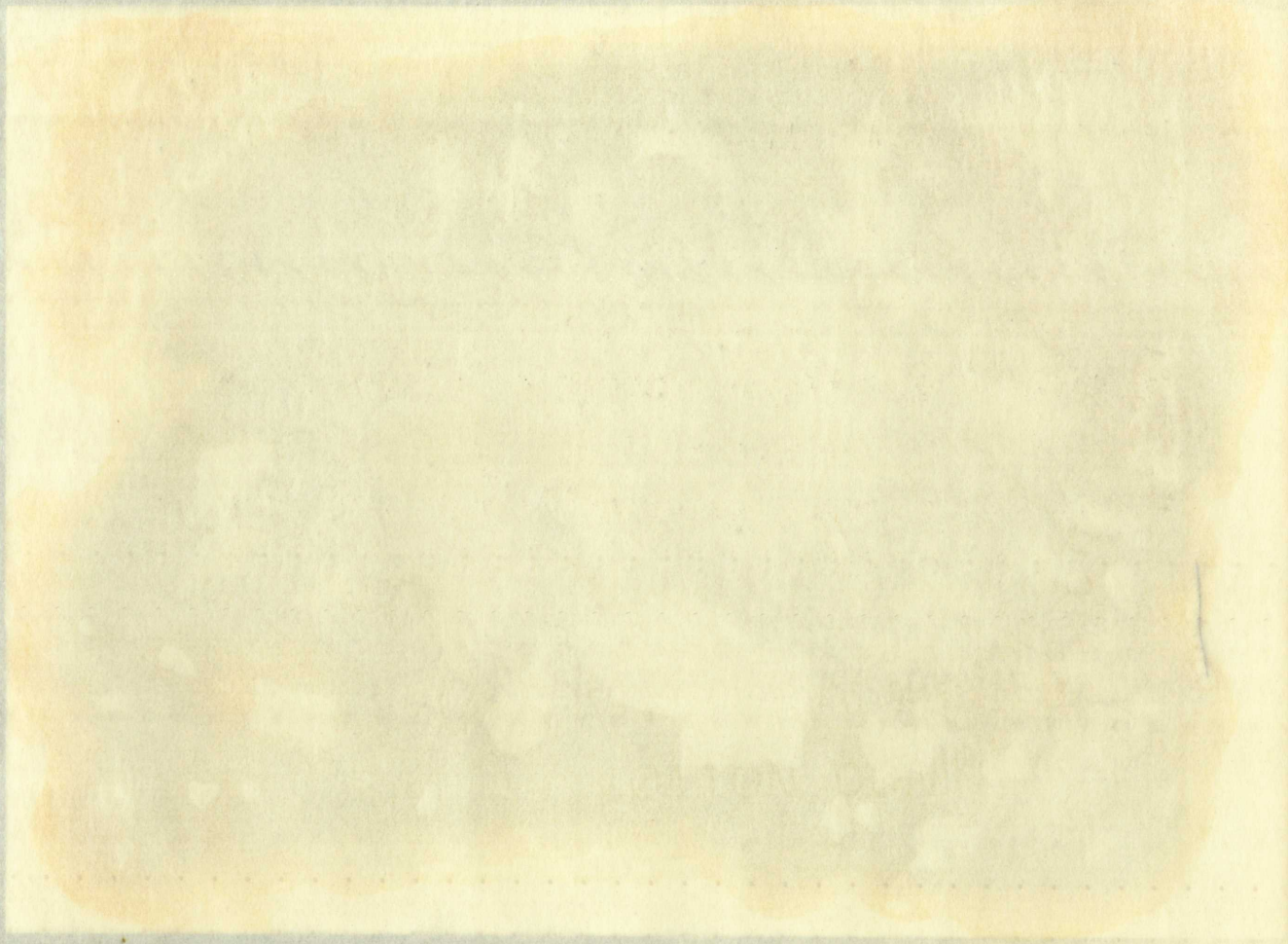


Figure 2 -- Lamination Curve



This projected approach is more complex than the present one and will be, perhaps, more fruitful. The simple approach was tried first because if positive results had been found the second approach would have been unnecessarily complex.

Another advantage in the second approach is that electrical stimulation will be used to locate the handedness area, and electrical desiccation will be used to produce a small lesion that will serve as a mark. After processing the brain, the mark will appear on those slides designated by electrical stimulation and lamination scanning will be performed under those marks. This approach has already been established as experimentally feasible. (Earp, 1959)

SUMMARY AND CONCLUSIONS

Ten rats were tested for preferential handedness, their brains removed, and the area in each hemisphere between white matter and caudate nucleus sectioned at 40 μ. These sections were stained by the Kluver-Barrera technique. The sections were investigated photometrically by measuring optical density in the white matter and layer VI of the cortical area concerned with handedness. The results of this investigation reveal no relation between density of these cortical areas and preferential handedness. Consequently, the hypothesis under test, that is, that the white matter and layer VI of the preferred hemisphere impede more light than those of the hemisphere controlling the non-preferred hand, is not supported.

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APPENDIX

APPENDIX

KLUVER-BARRERA STAINING METHOD

A. Make up a 0.1% solution of Luxol Fast Blue MBS by dissolving 1 gram of the substance in 1000 cc. of 95% alcohol. Add 5 cc. of 10% acetic acid. Filter before using. (This solution is very stable and may be used even after one year.)

B. Make up a 0.1% aqueous solution of Grubler's Cresyl-echtviolett or, if not available, a 0.25% solution of Coleman and Bell's cresyl violet. Before using, add 5 drops of 10% acetic acid to every 30 cc. of solution and filter. When using the Coleman and Bell product, heat solution before filtering.

Method for Frozen Sections

1. Cut sections at 40 microns and place in distilled water.
2. Immerse in 70% alcohol for 10 to 15 minutes.
3. Stain overnight (16 to 24 hours) in solution A at 40 degrees C. (For example, for staining 4 sections of the brain stem of a monkey, 20-25 cc. of solution A should be used and then discarded.)
4. Immerse in 95% alcohol and wash off excess stain.
5. Wash in distilled water. (Prolonged washing in water will not affect the staining.)

6. Begin differentiation by quick immersion in a 0.005% aqueous lithium carbonate solution.
7. Continue differentiation in several changes of 70% alcohol until gray and white matter can be distinguished. Care should be taken not to overdifferentiate.
8. Wash in distilled water.
9. Finish the differentiation by briefly rinsing in 0.05% lithium carbonate and then putting through several changes of 70% alcohol until there is a sharp contrast between the greenish-blue color of the white matter and the colorless gray matter. Care should be taken to rinse only briefly in the lithium carbonate solutions (3 to 5 seconds) since the final most delicate differentiation occurs in 70% alcohol.
10. Wash thoroughly in distilled water.
11. Stain from 1 to 2 minutes in Solution B (which should be carefully warmed before using).
12. Wash briefly in distilled water. (This step is necessary only when using the Coleman and Bell product.)
13. Differentiate in several changes of 95% alcohol.
14. Clear in a xylol-terpineol mixture.
15. Clear in xylol and mount.

IMPORTANT!

Special care should be taken to prevent loss or damage of this volume. If lost or damaged, it must be paid for at the current rate of typing.

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